Forage Genetics and Production

Nitrogen Cycling in Birdsfoot Trefoil-reed Canarygrass Pastures

M.P. Russelle, C. Sheaffer, C. Christians and B. Head, Jr.

Introduction

Because of their longer growing season, higher water use, and high inorganic N demand, perennial crops can significantly reduce nitrate leaching losses compared to annual crops. Research in humid regions has demonstrated that grazed forages lose more nitrate than mowed forages, due to the recycling of N by herbivores. Little is known about N cycling and loss in pastures under subhumid conditions. This information is needed to develop agricultural systems that are profitable and protect the environment.

We designed this experiment to answer the following questions: Is nitrate leaching likely on shallow midwestern soils under perennial forages? Does grazing increase leaching? How much N_2 fixation and transfer occur by birdsfoot trefoil?

Materials and Methods

Plots of birdsfoot trefoil (*Lotus corniculatus* L.) and a birdsfoot trefoil-reed canarygrass (*Phalaris arundinacea* L.) mixture were established on a Waukegan silt loam soil (Typic Hapludoll) in St. Paul, Minnesota. This soil formed in shallow (about 90 cm) loess over sand and gravel outwash. Because this soil is shallow, the nitrate leaching potential is high. We installed four 30-cm diam. drainage lysimeters in three replicates of each forage combination that were to be grazed and two lysimeters in those plots that were to be mown. Leachate was collected daily after every precipitation event until drainage was less than 1 mm. Nitrate concentration was determined by flow injection analysis.

The forages were grazed by ewes or were mowed four times per year in each of two years. Ewes grazed similar forages for 48 hours before entering the pastures. Ten to 25 ewes were used to remove forage from 185 m^2 plots within 24 to 48 hours and the six replicates were grazed in the same sequence each time (replicate one first, then replicate two, and so on). Forage samples taken before and after grazing were used to estimate dry matter consumption.

Symbiotic N_2 fixation and transfer were determined on a subset of samples using the ^{15}N isotope dilution technique and the difference method. In April 1997, 0.15 g $^{15}N/m^2$ was added to 1.5 m^2 microplots in six replicates of all treatments. Additional grass only microplots were maintained as controls using selective herbicides and hand weeding. New microplots were established in 1998. Herbage samples taken from these plots were analyzed for total N and ^{15}N concentration. The percentage of N derived from the atmosphere was calculated as $\%Ndfa = (1- [(atom\%excess_{fix} - atom\%excess_{con}/ atom\%excess_{con}]) X 100$, where the subscripts 'fix' and 'con' represent fixing and control plants, respectively, and atom%excess is the atom percent ^{15}N above natural abundance.

Shallow (0 to 15 cm) and deep (0 to 90 cm) soil cores were taken to evaluate inorganic soil N supply. Spatial distribution of inorganic soil N was determined by taking 91 (0 to 15 cm) cores per paddock at variable spacing (>5 cm). Soils were extracted with 2 *M* KCl and analyzed by flow injection.

Results and Discussion

Birdsfoot trefoil comprised a declining proportion of the sward throughout the study, presumably due to competition from weeds and reed canarygrass and to selective grazing pressure by the sheep (Fig. 1). Each year about 5.5 Mg/ha dry matter was consumed by the sheep or harvested by mowing.

Trefoil relied more on symbiotic N_2 fixation under mowing (> 95%Ndfa) than under grazing (67% Ndfa). This is likely due to increased inorganic N supply released by excreta from the grazing animals and we estimate that N derived from the soil was twice as large in the grazed than the mowed plots. Reliance on N_2 fixation was greater in mixtures (> 95%Ndfa) than in stands without reed canarygrass (68% Ndfa), presumably due to reduction in inorganic soil N supply available to the birdsfoot trefoil. As much as 10 kg N/ha apparently was transferred from the trefoil to the grass directly (i.e., not through the sheep) during one regrowth interval. Further research is needed to better quantify fixation and transfer rates where birdsfoot trefoil populations are not declining.

Soil inorganic N levels were low, except in small areas affected by excreta. There was no difference between spring and mid-season soil inorganic N levels. Mean topsoil nitrate concentrations were 17 mg N/kg in grazed trefoil, 7 mg N/kg in grazed mixtures, 3 mg N/kg in mowed trefoil, and 2 mg N/kg in mowed mixtures. Soil inorganic N was highly variable across the plot area (Fig. 2). Localized patches exceeded 120 mg nitrate-N/kg soil, but the vast majority of the area contained less than 20 mg N/kg in the topsoil.

Leaching losses were <1.5 kg N/ha annually, regardless of treatment. Nitrate concentration of leachate rarely exceeded the public drinking water standard of 10 mg N/L. In comparable soils, corn and soybean often lose 20 to 80 kg N/ha

annually. Thus, both grazed and mowed perennial forages appeared to protect ground water quality in this environment under the conditions of our experiment.

Conclusions

Little nitrate leaching occurred in this experiment and There was no difference between grazed and mowed forages. Consistent with other research conducted in the region, this suggests that grazing on fine-textured soils does not threaten ground water quality when fertilizer inputs are moderate. We are conducting other experiments to determine fertilizer N responses and losses in pastures and to evaluate nitrate leaching on sandy soils.

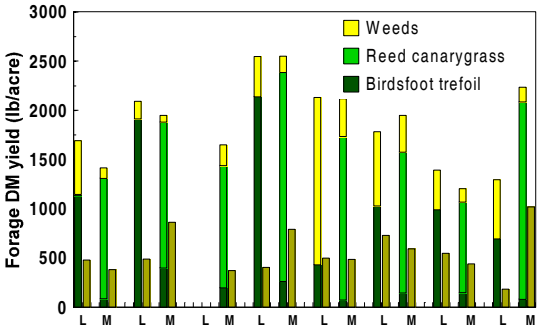

Figure 1. Pre-grazing (multiple-segment columns) and post-grazing (single-segment columns) pasture yield over two years. Treatments were birdsfoot trefoil only (L) or trefoil-reed canarygrass mixtures (M).

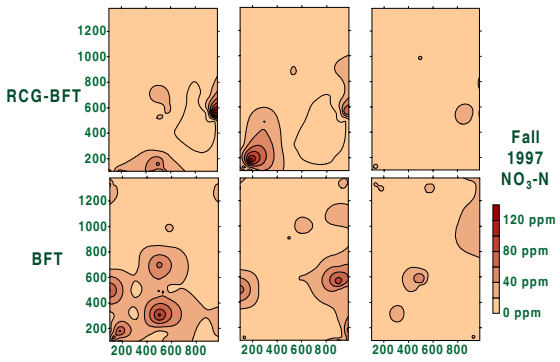

Figure 2. Kriged soil (0-15 cm) nitrate concentrations in six paddocks grazed by ewes. The lower three were birdsfoot trefoil; the upper three trefoil-reed canarygrass mixtures. Axes are distance from the SE paddock corner (cm).

Survey of Dairy Farmer Knowledge About Phosphorus

M. Schmitt, C. Hasthorpe, M.P. Russelle and J.M. Powell

Introduction

It is critical to understand current farm practices and farmer knowledge in order to develop appropriate agricultural research objectives and outreach approaches. For example, the role of phosphorus (P) in plant nutrition and environmental quality is well understood by researchers and has been a topic of private and public farmer training programs, especially in the past few years. What do dairy farmers understand about phosphorus? How does this understanding relate to local program efforts or farm size? Our objective was to answer these and other questions.

Materials and Methods

We developed a four-page questionnaire of multiple choice, true-false, fill-in-the-blank, and open-ended questions. Sections included 'Soil testing and commercial fertilizer use,' 'Phosphorus information,' 'Manure management,' 'Your views,' and ' Your farm.' The questionnaire was sent by mail to all dairy farmers in four counties in southeastern Minnesota and one county in west central Wisconsin in fall 1999. A letter signed by the county extension educator accompanied the survey. A reminder postcard was sent five weeks later.

Results and Discussion

Response rate of valid returns was 21% (197 respondents). This rate is considered acceptable based on the generic mailing lists used, the lack of incentives for completion, no follow-up phone calls, and the overall inundation of surveys to all dairy farmers from companies, the Agricultural Statistics Service, and universities.

Median number of cows milked was 57, similar to results from several recent surveys in both states. Acreage of corn and soybeans increased with farm size, whereas alfalfa acreage declined. Even with the large farms, alfalfa comprised an average of 30% of the acreage.

Over two-thirds of the farmers said they hauled manure daily or weekly. This, too, is consistent with other surveys, and highlights the need for research and education aimed at helping these farmers manage manure. Farmers that relied on daily or weekly manure hauling had a median herd size of 50 cows in both states. In contrast, farmers that applied manure only once or twice per year had median milking herds of 51 cows in Wisconsin but 85 cows in Minnesota. This reflects the larger number of large herds in the Minnesota survey and perhaps the more widespread use of manure storage facilities in typical herds in Wisconsin.

Farmers with small herds hauled manure less far than those with larger herds. Average distance of manure hauling was about 1 mile for 40 or fewer milking cows, 1.4 miles for 41-60, about 2 miles for larger herds up to 200 head, and 4 miles for milking herds of more than 200 cows. For the same herd sizes, the acres receiving manure were 49, 75, 170, and 325 acres. The average annually manured land area to animal unit (milking herd only) ratio was 1.1 across all farms. This ratio must be interpreted cautiously, as we have no information on the numbers of other livestock on the farm, although we do know that three-quarters of the respondents have beef cows and approximately one-half raised feeder cattle.

Dairy farmer knowledge about P appears to be both limited and faulty in the majority of cases. Although most farmers say they use soils tests and follow fertilizer recommendations "exactly" or "somewhat closely", most (74%) did not know the appropriate soil test P level for optimum corn production. Only 15% knew P does not move appreciably in soil. One in six knew that P affects surface water quality, but the remainder mistakenly thought P also affects ground water quality or did not know the environmental impact of P. About 40% knew that P concentration is lower than N in both manure and corn, but the remainder incorrectly answered either that P and N levels are equal or that P exceeds N. There was no apparent difference in farmer knowledge between the states.

Whereas only one-third of the farmers said there is "too much fuss over nutrients, fertilizers and the environment," about 75% did not want more regulations on manure management. Most reported that surface water on their farms was as good or better than it was 10 years ago. Although it is conceivable that they correctly evaluate conditions on their farms, this response conflicts with assessments of surface water quality in both states.

Conclusion

There is a great need for improving knowledge about P among our dairy farmers. Inaccurate and incomplete knowledge may contribute to poor on-farm management of nutrients. Nutrient management plans, which are required in many areas, are more likely to be correctly implemented if farmers understand the reasons for limits on application rates, field selection for manure application, etc. In addition, research to improve management of daily or frequent manure hauling scenarios and implementation of improved manure storage and handling processes are needed on the vast majority of dairy farms we surveyed.

Forages Show Little Response to High Soil P Levels

M. Russelle, M. Schmitt, J.M. Powell and C. Hasthorpe

Introduction

Optimum soil test levels for phosphorus (P) are typically between 20 and 30 mg/kg (ppm) for soil extracted with the Bray P1 procedure. Below that soil test P (STP) level, crops are expected to respond positively to P applications, whether from manure or fertilizer materials. Above that level, little or no yield response is expected. Unlike the situation with nitrogen and potassium, there is little evidence that crops accumulate P in 'luxury' amounts that is in excess of concentrations in the plant needed for optimum yield.

There is considerable evidence that soils on dairy farms have increasing STP levels. These increases are related to repeated fertilizer and manure applications at rates that exceed crop P removal. Regulations are being promulgated to limit manure application on high-testing soils, because runoff of P decreases surface water quality. Numerous studies have shown that manure application is nonuniform; thus, we expect that STP levels will also vary widely, even on fields that have received fertilizer and manure over several decades. This study was conducted to determine 1) the variability of STP levels on dairy farm fields; and 2) whether alfalfa or corn showed apparent responses to STP (and associated changes related to manure applications).

Methods

During the 1999 growing season, we sampled 2 to 4 fields of alfalfa and corn on each of 8 dairy farms in Olmsted Co., MN, and Trempealeau and Pierce Co., WI. Soils were all loess-derived silt loams. Five to 8 randomly selected areas in each field were sampled at the first three alfalfa harvests, at tasselling, and after physiological maturity in the corn. Alfalfa herbage was removed from $1m^2$ and corn plants were sampled from 6 m of row. Ears were separated from stover at maturity. Dry weight was determined, the samples were finely ground, and total P was measured after dry ashing.

Samples were taken from the top 15 cm of soil in each plant sampling area at every sampling time. These were air dried, ground, and analyzed for Bray P1 extractable P. A subset of 45 samples from alfalfa fields was analyzed for extractable soil K and total plant K. Regression analysis was used to determine whether significant relationships existed between variables.

Results and Discussion

STP levels varied widely within fields, especially as median STP level increased (Fig. 1). These results indicate that it is particularly important to take numerous samples from fields with near optimum (dashed line) to above optimum STP levels, in order to adequately estimate the median STP level. It also indicates that it may be worthwhile to consider site-specific fertilizer or manure applications, because fields that had generally adequate STP levels still had areas with below optimum STP.

There was no effect of STP on corn dry mass at tasselling (mean=5 Mg/ha), but a slight increase in plant P concentration was found (although a quadratic equation explained only 29% of the variation in the data). No effect of STP was noted on mature corn grain or stover yield (mean grain dry matter yield was 6.86 Mg/ha) nor on corn P concentration or P uptake.

STP had no effect on first cut alfalfa yield (mean = 3.1 Mg/ha), but second and third cut yields were slightly higher with increasing STP. Most of this latter response was limited to the normal STP response range (< 30 ppm). In contrast, neither second nor third cuttings showed a plant P concentration effect of STP (mean second cut = 0.34% P; mean third cut = 0.38% P), whereas first cut alfalfa increased from about 0.3% P at low STP to 0.45% P at 100 ppm STP and above. Although these samples represent forage without the typical dry matter losses during hay or haylage making operations, P concentrations significantly exceeded the NRC book value (0.29% P) for alfalfa hay. Five of seven alfalfa hay samples procured on these farms exceeded the NRC book value (mean = 0.36% P); two were lower (mean = 0.28%P), substantiating research results by others in Wisconsin and elsewhere.

As with corn, total P removal by alfalfa over three harvests was small (Fig. 2). Although summer harvests yielded less than normal due to drought and leafhopper damage, farmers should not expect crops to remove large amounts of P. For example, an annual yield of 8 Mg/ha and a P concentration of 0.4% remove only 32 kg P/acre. Avoiding excessive P accumulation in soil is the preferred option, and this can be achieved by applying manure based on the P requirements of crops.

There was a high correlation between STP and soil test K (STK) levels. This was not surprising, as we expect STP to be related to past manure applications. As a result of this correlation, alfalfa contained considerably higher K concentrations at high STP (STK) levels. Most forage samples were below the NRC book value at STK levels lower than the optimum, whereas most forage K concentrations exceeded NRC book value and recommended dietary levels at higher STK.

Conclusion

This research found little indication of luxury P uptake, except in the first alfalfa harvest and effectively no yield response to higher than optimum STP (or STK) levels. Relatively little P can be removed in harvested forage, so using crops to remove excess STP will be very slow. In addition, high P-testing soils may also produce hay that contains too much K to be safely fed to dry or close up cows.

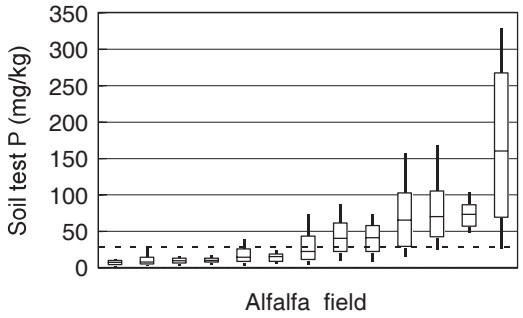

Figure 1. Box plot of STP levels in 13 alfalfa fields. Minimum and maximum values are indicated by the vertical lines; the box indicates the 25, 50, and 75% quartiles.

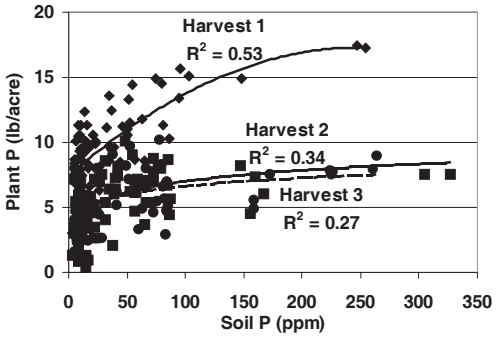

Figure 2. Total P uptake by alfalfa in each of 3 harvests during 1999.

Dairy Diet Effects on Phosphorus Cycles of Cropland

J.M. Powell, Z. Wu and L.D. Satter

Introduction

Excessive soil nutrient accumulation, the transfer of nutrients through surface and subsurface runoff, and pollution of our lakes and streams are environmental challenges facing the dairy and other livestock industries. To remain economically viable, many dairies are increasing herd size and importing more and more feed. In these systems, the repeated importation of feed and fertilizer P has resulted in large positive P balances and the subsequent build-up of soil test P levels, much above what is needed for optimal crop yields. The control of P inputs is of prime importance in reducing P runoff losses and the eutrophication of our lakes and streams (Sharpley et al., 1994). At present, our knowledge base remains incomplete with respect to the environmental implications of important nutrient management options, such as optimal feeding of nutrients, proper handling, storage and land application of manure, and cropping patterns that minimize nutrient buildup and losses. Recent research at the DFRC (Satter and Wu, 1999) has shown that dietary P can be reduced by 25 to 30%, which reduces manure P by a greater percentage, without sacrificing milk production or quality. The objective of this study was to show how reductions in dietary P affect the amount and forms of P excreted in manure, the land required for recycling manure P through crops, and the ability of a farmer's land base to recycle manure P in view of new legislation that limits the land application of manure based on crop P requirements (USDA-USEPA, 1999).

Methods

Feces were obtained during the mid lactation period (24 weeks) from cows fed various levels of dietary P (Satter and Wu, 1999) and analyzed for total and water-soluble P content. We used a typical cropping pattern of a dairy farm in Wisconsin (42% of the cultivated area devoted to alfalfa, 33% to corn grain, 8% to soybean and 6% to corn silage), the average P uptake (30 kg ha^{-1}) of this cropping pattern, and guidelines that limit manure applications to the amount of crop P removal, in an analysis of how dietary P affects the cropland area needed for recycling manure P through crops. To illustrate the effect of diet P supplementation on a farm's ability to recycle P, we took an example of a dairy farm that typifies the field distribution of soil test P (Proost, 1999) and calculated the farm's capacity to store additional P in the 0-15 cm soil depth until all fields reach exessive soil test P levels.

Results and Discussion

After feeding, dairy cows secrete P in milk and excrete P in feces. Little P is excreted in urine. The addition of inorganic P to dairy diets (x = daily grams of P intake) results in greater fecal P excretion (y = g P in feces) in the following manner: for total P in feces y =.60x + .241, R-sq. = .67, for water soluble P in feces y=.21x - 2.28, R-sq. = .55. For the range of dietary P levels used in this study, water-soluble P accounted for approximately 30 to 35% of the total P in dairy feces. Water-soluble P consists of inorganic orthophosphate that is readily available for algae uptake, and therefore, is linked to eutrophication (Krogstad and Lovstad, 1991).

The proposed shift from a N-based to a P-based strategy for land application of manure (USDA-USEPA, 1999) may profoundly affect nutrient management on dairy farms. Many dairy farms have fields that test "high" or "excessive" in soil test P (Proost et al., 1999). If the amount of manure applied to cropland is restricted to crop P removal, the supplementation of the dairy diet with inorganic P can increase dramatically the land requirement for recycling manure P (Table 2). Approximately 0.60 ha would be required to recycle manure P excreted by a lactating cow (9,100 kg milk over 305 d) fed a diet containing 3.8 g kg^{-1} P. The annual addition of 6.8 kg of supplemental P to raise dietary P from 3.8 to 4.8 g kg^{-1} (Table 1) for a single lactating cow increases the cropland area needed for recycling manure P by 0.23 ha, or 38% (Table 2).

Recycling manure nutrients through crops is critical to proper manure management. However, the effective recycling of manure nutrients through crops presents many challenges. For example, the manure of ruminant livestock typically has an average N:P ratio of 4, while the N:P requirement of major grain and hay crops is approximately 7 to 8. Dietary P levels can exacerbate the difference in N:P ratios between crops and manure (Fig. 1). This means that manure from cows fed excessively high P diets, when applied to cropland in amounts to meet a crop N demand (i.e. manure application to fields having low or medium levels of soil test P) will increase soil test P much quicker than the application of manure derived from cows fed diets that provide adequate, but not excessive, amounts of P. Reducing dietary P would not only reduce manure P levels but would also improve the N:P ratio of manure to more nearly match N:P ratio of plants (Fig. 1).

A farmer's decision on how much supplemental P to feed would profoundly affect how many cows and for how long the farm could support milk production before all fields attain excessive levels of soil test P (Table 3). By supplementing only minimal amounts of P to meet the cow's requirement (3.8 g kg^{-1}), and eliminating fertilizer P altogether, the case-study farm can continue to support 90 lactating cows indefinitely. The addition of dietary P supplements in excess of the cow's requirement results in manure P in excess of crop P requirements, and reduces the number of cows the farm can support and/or the number of years before all fields reach excessive soil test P levels.

Conclusions

This study has shown that the luxurious use of dietary P supplements increases dramatically the land required for recycling manure P. In situations where cropland available for manure spreading is limited, dietary P supplements build-up soil test P thereby reducing the number of years a farm can support dairy production before all fields attain excessive soil test P levels. A strategy of reducing dietary P supplements would benefit dairy producers, since both feed costs and the amount of manure P that has to be recycled would be reduced. We should seek a more holistic understanding of how nutrient management in one production component (e.g. feed) affects nutrient cycling in other production components (e.g. soils and crops).

Stronger partnerships among researchers, extension agents, feed, and fertilizer consultants will be necessary to provide unified approaches to technical assistance and educational programs aimed at influencing nutrient management decisions of farmers.

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Table 1. Annual phosphorus fed and excreted in feces by a lactating cow.[†]

Dietary P level	Supplemental P	Fecal P
g kg^{-1}	kg cow^{-1} $year^{-1}$	
3.5[‡]	0	18.9
3.8[§]	2.5	21.4
4.8	10.6	29.6
5.5	16.4	35.3

[†]Assumptions: Cow is producing 9100 kg of milk per 305 d, and consuming 22.5 kg dry matter per day, or 6863 kg per 305 day. Milk contains 0.9 g kg^{-1} P, no net change in body P content of the cow.
[‡]May be marginally deficient in P for very high producing cows.
[§] Recommended level of dietary P (Satter and Wu, 1999).

Table 2. Land requirement for recycling fecal P excreted by a cow fed various dietary P levels.

Dietary P level	Land area needed to recycle fecal P	Change in land area due to diet P supplementation
g kg^{-1}	ha[†]	%
3.5	0.63	0
3.8	0.71	13
4.8	0.99	57
5.5	1.18	87

[†]Annual cropping system comprised of 47% is alfalfa, 37% corn grain, 9% soybean and 7% corn silage having harvested dry matter of 11.2, 7.4, 2.9 and 17.2 Mg ha^{-1}, respectively, and P removal of 30 kg ha^{-1}

Table 3. Effect of dairy diet P supplementation on a farm's ability to store soil P.[†]

Dietary P level	Fecal P	Years to attain excessive Bray1 P in all fields
g kg^{-1}	kg cow^{-1} yr^{-1}	
3.5	18.9	indefinite
3.8	21.4	indefinite
4.8	29.6	11
5.5	35.3	6

[†]Example of a typical dairy farm (Proost et al; 1999) that has 68.8 ha that can receive up to 6347 kg P (i.e. 721 mg kg^{-1} Bray1 P) before all fields attain excess Bray1 P levels. Assumes a stocking rate of 90 lactating cows and an annual crop P removal of 30 kg ha^{-1}.

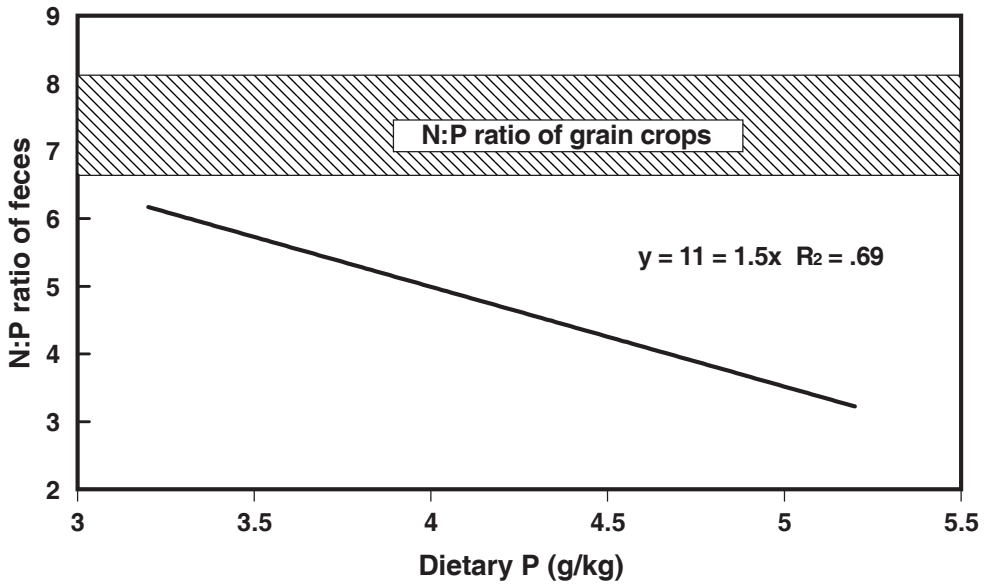

Figure 1. Dairy diet effect on N:P ratio of feces.

Measurement of Nitrogen Availability from Repeated Manure Applications

J.M. Powell and K.A. Kelling

Introduction

Most dairies in the Great Plains, Cornbelt and Northeast regions of the U.S are land-based. They produce most of their feed and use their land base to recycle manure nutrients through crops. The recycling of manure nutrients through crops is critical to proper manure management. Although it has been shown that proper manure management can be profitable through reduced fertilizer costs, many farmers do not credit the nutrients contained in manure (Nowak et al., 1997). In areas where manure has been land-spread, many farmers continue to apply fertilizers in sufficient quantities for attaining desired crop yield. The lack of manure nutrient crediting by farmers may be due to many factors that make manure an undependable source of plant nutrients. These may include differences in soil fertility levels where manure application experiments have been conducted, inherent shortcomings of the classical, indirect methods used to estimate manure nutrient mineralization and crop uptake, and other problems.

Estimates of manure nutrient availability to crops, otherwise known as "nutrient credits", are currently single nitrogen (N), phosphorus (P) and potassium values given for the type of manure applied (solid or liquid), method of application (incorporated or not) and frequency of application. The lack of manure nutrient crediting by farmers may be due to high variability associated with indirect methods, such as the "difference method" and the "fertilizer equivalent approach" used to measure manure nutrient availability. For example, using the difference method and the fertilizer equivalent approach, from 12 to 63% of dairy manure N and from 12 to 89% of dairy manure P have been estimated to be taken up by corn during the first growing season after application (Motavalli et al., 1989; Klausner et al., 1994). Nutrient availability in the second and subsequent years can be even more difficult to predict. More accurate estimates of manure nutrient availability are needed if we are to expect farmers to improve manure management.

The stable isotope ^{15}N has been used extensively to evaluate the availability of fertilizer N to crops (Hauck and Bremner, 1976; Menzel and Smith, 1984). The use of ^{15}N in nutrient cycling studies involving the manure of ruminants has been limited (Sorensen and Jensen, 1998). Most studies have involved the labeling the ammonium in manure. These studies assume that manure ammonium is the only manure N component that becomes available and is taken up by a crop during the season following manure application (Paul and Beauchamp, 1995; Sorensen and Jensen, 1995). However, only 30 to 60% of dairy manure N is ammonium (Paul and Beauchamp, 1995; Motavalli et. al., 1989). The chemical composition and mineralization rates of organic N in animal manure are not well understood. Rumen microbial products and other endogenous, organic N forms in feces may make a significant contribution to crop N requirements. The objectives of this field study are to examine (1) the effect of manure application rate and frequency on manure ^{15}N uptake by corn silage and (2) to compare manure ^{15}N uptake to N uptake estimated by the difference method and the fertilizer equivalent approach.

Methods

Fecal and urine N components were enriched in ^{15}N following procedures outlined by Powell and Wu, (1999) and Powell et.al, (1999). A field trial was initiated on a Plano silt loam (fine-silty, mixed, mesic: Typic Agriudoll) at the West Madison Experiment Station during the spring of 1998. Treatments include six fertilizer N levels (0, 50, 100, 150, 200 and 300 kg N ha^{-1}), three manure rates (0, 80 and 160 kg mineral N ha^{-1}) and three manure application intervals (every 1, 2 or 3 years). A strip-plot design was used with fertilizer N and manure rates applied to strips and manure intervals applied to blocks. There are 4 replications of each treatment.

Manure intervals "every 1 year," "every 2 years," and "every 3 years" are established, and continuation of the intervals are as follows:

1998	1999	2000	2001	2002	2003
$I_{1(1998)}$	→	→	→	→	→
$I_{2(1998)}$	$I_{2(1999)}$	$I_{2(1998)}$	$I_{2(1999)}$	$I_{2(1998)}$	$I_{2(1999)}$
$I_{3(1998)}$	$I_{3(1999)}$	$I_{3(2000)}$	$I_{3(1998)}$	$I_{3(1999)}$	$I_{3(2000)}$

Where the first subscript number following the "I" refers to the manure interval (i.e., every 1, 2 or 3 years), the second subscript in parentheses refers to the year the interval was established. For example, $I_{1\,(1998)}$ refers to manure applied every year, this interval established in 1998 (and continuing throughout the project); $I_{2\,(1998)}$ refers to manure application every 2 years, this interval established in 1998 (receiving manure every 2 years thereafter); $I_{2(1998)}$ also refers to manure applications every 2 years, but this interval is established in 1999, etc.

Three approaches are used to estimate manure N uptake by crops: (1) difference method, (2) fertilizer-equivalent and (3) the ^{15}N approach. The difference method calculates the difference in total N uptake between manured and non-manured plots. The fertilizer equivalent of manure is the amount of fertilizer N required to achieve the same yield and/or N uptake achieved with manure. For the ^{15}N approach, manure enriched in ^{15}N is applied to 3.48 m^2 subplots within blocks of manure N application rate of 160 kg ha^{-1}. All ^{15}N manure is hand applied and incorporated with a disk plow.

Corn (for silage) is grown continuously in all plots and managed according to standard practices.

Results and Discussion

First year results of the field trial to compare manure N uptake using ^{15}N-labeled manure to the difference method and fertilizer equivalent approach are given in Table 1. Average manure N uptake by corn silage was similar for the ^{15}N and difference methods, which were approximately half the estimates of the fertilizer equivalent approach. Lower estimates of crop N uptake using ^{15}N than the fertilizer equivalent approach have been reported (Thomsen et.al., 1997; Jensen et.al., 1995). Perhaps the most remarkable result of our first year comparisons is the much narrower range of N uptake values associated with ^{15}N-labeled manure than with the difference or fertilizer equivalent methods. The continuation of this trial and lower variance associated with ^{15}N may greatly improve the precision by which we can estimate manure N uptake by crops. This result may reduce the risk associated with nutrient crediting and improve manure management.

Conclusions

The results of this study should have several theoretical, as well as practical implications for improving environmental impacts of dairy manure management. The proposed ^{15}N technique may provide a tool for better understanding nutrient flow in various components of the animal-feed-manure-soil/crop-environment continuum. Combined with the information gained from the ^{15}N labeling of manure (see Research Summary on pp. 68-70 to) we will be able to evaluate the conversion of inorganic fertilizer N into forage N (alfalfa and corn silage), inorganic and forage N into dairy urine N and fecal N components, and the subsequent recycling of dairy manure N components through soils back into forage N. The field trial should continue for a least 6 years in order to observe manure N dynamics in 3 replications of plots where manure has been applied every 2 years and in 2 replications of plots where manure has been applied every 3 years. The long-term nature of the trial and the use of ^{15}N-labeled manure and fertilizer N should provide opportunities for comparing direct and indirect measurements of manure nutrient dynamics under various manure management regimes. This information could increase our confidence in manure nutrient availability to subsequent crops and provide the basis for developing alternative, economically viable and environmentally sound manure management practices.

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Table 1.Direct (^{15}N) and indirect (difference method and fertilizer equivalent) estimates of manure N uptake by corn silage.

		Methods of estimating manure N uptake		
Measure of manure N availability		^{15}N-labeled manure (n = 12 plots)	Difference method (n = 4 plots)	Fertilizer equivalent (n = 4 plots)
N uptake (kg/ha)				
	mean	27	30	59
	range	10 to 55	-83 to 130	-104 to 273
% of N applied				
	mean	13	16	31
	range	5 to 27	-43 to 67	-54 to 141

Brown midrib (bm) Mutations in Corn

J.M. Marita, W. Vermerris, J. Ralph and R.D. Hatfield

Introduction

A brown midrib is the characteristic phenotype of a series of mutants in corn, sorghum, and pearl millet. There are four *brown midrib* (*bm*) mutants know in maize (*Zea mays* L.). These mutants, *bm1*, *bm2*, *bm3*, *bm4*, all have reddish-brown vascular tissue in the leaves and stems compared to a normal white-yellow coloring. This change in midrib coloring is correlated with differences in lignin content and/or composition. Lignin is a complex polymer of phenylpropanoid units found in vascular plant cell walls and is important for providing mechanical support, water transport, and physical barriers against pests and pathogens. The *bm* mutants of maize are of interest because of their potentially higher nutritional value as forage, presumably a result of a lower lignin content and resulting differences in cell wall structure. This work examines the changes in lignin content and cell wall structure between stem sections of all four *bm* mutants (near-isogenic lines within A619 inbred corn background), a double *bm1*/*bm2* mutant, and wild type (A619 inbred) corn plants.

Methods

Stems from field grown plants were obtained from Dr. W. Vermerris, University of North Carolina in Raleigh, NC. Cell walls were isolated from ground stem sections following the removal of soluble phenolics, carbohydrates, and other components by successive extractions with water, methanol, acetone, and chloroform extraction. Samples were dried and analyzed for lignin, neutral sugars, and total uronosyls.

Results and Discussion

Based on previous studies, the *bm1* mutation results from changes affecting cinnamyl alcohol dehydrogenase (CAD) and typically has a reduced lignin content in mature plants. The *bm2* mutation may reduce lignin up to 30% but the exact mutation lesion is not known. *Bm3* mutations are associated with a decrease in O-methyl transferase (OMT) activity and may reduce lignin content up to 12%. Lignin isolated from *bm3* mutants contains 5-hydroxyguaiacyl subunits and exhibits a reduction in syringyl units. The bm4 mutation shows a reduction in the level of syrinaldehyde and a reduction in lignin content by as much as 8%. In addition to the four single gene mutations, a double-*bm I*/*bm2* mutant was analyzed to examine the possible (additive) effects of multiple gene mutations. All *bm* corn mutations examined in this study were in near-isogenic lines (NILs) allowing direct comparisons between all mutant plants. Previously, *bm* mutants were compared to normal (wild type) plants with each study analyzing *bm* mutations in different genetic backgrounds.

In this comparison, lignin was measured as the recovery of an acid insoluble residue. Only two *bm* mutations (*bm3* and *bm1*/*bm2*) exhibit a reduction in lignin compared to wild type (A619) and these differences are not substantial (Table 1). *Bm2* (13.1%) and *bm4* (13.8%) mutants contain relatively the same amount of lignin as wild type (13.0%), but *bm1* (16.0) has slightly more lignin than wild type. Cell wall composition was generally the same among all corn stem sections (Table 1) specifically cell wall neutral sugars. The corn stem tissues contained between 47 to 50% cellulose (measured by glucose) and 22 to 27% xylan (measured by xylose) with low substitution with arabinose (13.5 to 14.2%). These results do not agree with prior studies examining the effects of *bm* mutations on lignin where, similar structural effects across *bm* mutations among different genotypes was observed but the extent of change varied. Presumably this can be attributed to the multiplicity of genes for each lignin biosynthetic step in corn. Furthermore, our analyses of the *bm1/bm2* mutant do not show an additive or deleterious effect from the double gene dose although a slight reduction in lignin exists. This data suggests that the cell wall composition like lignin of the *bm* mutants has not undergone the dramatic structural changes anticipated or reported previously, and the genetic background of the plant material may influence the effect of the *bm* mutation on cell wall composition.

Table 1. Cell wall composition of wild type and *bm* mutants of maize in A619 background.

	mg/gram cell wall					
Corn lines	Lignin	Uronosyls	Arabinose	Galactose	Glucose	Xylose
A619 (inbred)	130.0 ± 3.0	41.3 ± 2.0	36.7 ± 0.6	7.0 ± 0.1	479.1 ± 5.4	226.6 ± 3.2
bm1	159.9 ± 43.0	48.9 ± 2.0	34.4 ± 0.1	6.8 ± 0.2	471.8 ± 20.3	252.9 ± 0.4
bm2	130.8 ± 4.0	43.2 ± 2.0	32.6 ± 0.3	6.5 ± 0.1	469.1 ± 4.2	252.0 ± 4.9
bm3	103.9 ± 2.0	36.7 ± 0.3	32.7 ± 1.0	6.9 ± 0.4	491.2 ± 3.5	262.1 ±3 .2
bm4	137.5 ± 1.0	41.0 ± 2.0	34.4 ± 0.6	6.7 ± 0.2	485.7 ± 7.1	242.0 ± 0.3
bm1/*bm2*	117.6 ± 3.0	41.5 ± 0.3	33.0 ± 0.5	6.5 ± 0.2	472.5 ± 9.7	244.9 ± 3.9

In-Field Wet-Fractionation of Transgenic Leguminous Herbage

R.G. Koegel, R.J. Straub and M.E. Boettcher

Introduction

The petroleum industry has, for many years, fractionated crude oil into a variety of products each of which is more useful and valuable than the original commodity. ARS and University of Wisconsin researchers at Madison, Wisconsin believe that they can profitably fractionate herbage from plants like alfalfa or forage soybean to create new products and increase demand for these crops. The first step is separation of the fresh herbage into a juice fraction and a fibrous fraction, a process known as "wet fractionation". Products from the juice fraction include food-grade and feed-grade protein concentrates, carotenoids, anti-oxidants and industrially valuable enzymes. An example of the last is the enzyme phytase produced in transgenic alfalfa. When fed to poultry or swine, this enzyme allows the animals to use more of the phosphorus in their grain-based rations and to reduce the phosphorus content of the manure.

Products from the fibrous fraction include, in addition to cattle feed: chemical feedstocks, filter mats for removing pollutants from water, enzymes produced by growing fungi on the fiber, and building materials.

Methods

In the past, when wet fractionation was carried out, either on a research basis or as a commercial venture, it was done in a central processing facility. This meant that herbage, containing around 80% water, had to be transported from the field to the facility and waste liquid had to be dehydrated or transported back to the field as a liquid fertilizer. This led to high transportation and energy costs as well as delays in processing. The approach used in this work during the summer of 1999 was to carry out wet fractionation at the field where the crop was grown to eliminate some of the transportation cost and delays between cutting and processing the crop. In this case the field-side process was only carried through the juice/fiber separation step with the juice transported to a local biotech company for separation of the target substance. Ideally, much of this separation process would also have been carried out at the field to minimize liquid transport.

The processing sequence was (1) cutting of the crop, (2) transport to the processing equipment, (3) milling to rupture plant cells, (4) juice expression, and (5) loading of juice and fiber onto their respective transport vehicles.

Commercially available equipment was used as much as possible. Cutting and transport of the crop was carried out with a forage harvester towing a self-unloading wagon. Milling was accomplished with a hammer mill, which had been extensively modified. Juice expression was carried out in a 16-inch diameter screw press, a continuous press consisting of a slowly rotating screw inside of a perforated cylindrical housing. As the screw transports the milled herbage through the housing, pressure is built up against a restriction at the housing outlet, causing juice to be expressed. Conveyors for the solid material and a pump for the juice completed the equipment.

The hammer mill had been originally designed to comminute brittle, free-flowing grain using rotating hammers to crack and drive the grain through holes in a screen. In this application, however, the hammers were used to impact the herbage multiple times as it passed through the mill for the purpose of cell rupture without necessarily reducing particle size of the resilient, fibrous herbage. This was done by replacing the screen with a solid metal concave which described a 180° arc just beyond the tips of the rotating hammers. This caused the herbage to be impacted multiple times as it traveled along the 180° arc of the concave from inlet to outlet of the mill. An adjustable restriction at the outlet could be narrowed to increase the degree of impacting. Since, unlike grain, the herbage is not free flowing, an oscillating inlet hopper was used to shake the herbage into the mill. A conveyor elevated the herbage from the self-unloading wagon into the mill and the milled herbage dropped by gravity from the mill into the press. Juice flowed from the press into a tank truck, and the fibrous fraction, which was not being used in this instance, was conveyed to a spreader for return to the field. A 60 horsepower and a 40 horsepower electric motor drove the mill and the press, respectively. Electricity for these and for various conveyors and pumps were supplied by a 120-kilowatt diesel-powered generator.

Results

Some representative values for the operation follow:

Initial moisture content of herbage	81%
Final moisture content of fiber	69%
Herbage throughput (fresh weight) tons/hour	6.4
Juice flow rate tons/hour	3.5
Power requirements:	
Mill horsepower	40
Press horsepower	25
Total horsepower	65
Specific energy hr/ton	10.2 hp-
	7.6 kwh/ton
Energy cost @ $0.10 per kwh	$0.76/ton

Conclusion

This operation demonstrated the feasibility of in-field wet fractionation, albeit at a relatively modest scale. Since, in this case, further separation of the juice was not carried out in the field, the benefit of reduced transportation cost was not fully realized.

This stationary field-side processing operation is considered an evolutionary step in the development of a mobile field processor, analogous to a combine, which would cut the crop and wet fractionate it concurrently with juice being off-loaded periodically to a field-side unit for further processing this would result in significant reduction in the weight to be transported over the road. Such a mobile processor would also provide the option of returning the fibrous fraction to the stubble for further field drying, where desired.

Continuous Wet Fractionation Press with Multiple Cone Chamber

R.G. Koegel, R.J. Straub and K.B. Chase

Introduction

Wet fractionation of forage crops, the separation of fresh herbage into juice and fiber fractions, makes possible a number of non-traditional, value-added products. Products of the fiber fraction include: chemical feedstocks, filter mats for removal of pollutants from water, enzymes produced by growing fungi on the fiber, and building materials. Juice products include food-grade and feed-grade protein concentrates, carotenoids, anti-oxidants and industrially valuable enzymes (from transgenic plants).

Juice expression has most frequently been carried out using screw presses. This type of press has a number of advantages including reliability, ability to achieve good juice yields, and adaptability to a wide variety of feedstocks. On the other hand, its throughput relative to size and weight are low, while specific energy requirement is high. Both of these are important disadvantages, especially for a mobile/agricultural operation. Consequently an evaluation of several alternative press configurations was carried our to determine if they might have advantages in specific weight and specific energy requirement.

Methods

The press evaluated had a chamber bounded by six conical perforated rollers (Fig. 1). The cone axes converged toward the chamber axis from the large (input) ends of the cones to the small (outlet) ends, so that the chamber cross-section decreased in area from inlet to outlet. In addition the cone axes were skewed (Fig. 2), so that when the material is introduced into the chamber inlet and all cones are rotated in the appropriate direction, it is simultaneously rolled and moved from the inlet to the outlet end. While traversing the chamber the material is compressed and liquid expressed through the perforations in the cone surfaces. Since this press involves only rolling contact, while the screw press involves sliding contact, it appeared possible that the former might have a lower power requirement.

The press which was evaluated had six cones tapering from six inches to three inches in diameter over a 24 inch length. It was considered to be about one-quarter scale compared to a full operational press. It was fed by a plunger reciprocating at the bottom of a supply hopper. This plunger packed material into the inlet end of the press, approximately doubling its density and expressing a limited amount of juice.

After preliminary evaluation with macerated alfalfa, a small diameter central drain was added, the axes of the drain and chamber being coincident. This drain was found to improve extent and uniformity of liquid expression, but to impede movement of material through the chamber. This problem was overcome by adding helical flighting to the drain and causing it to rotate as an auger.

Results

The press was effective in expressing juice. The moisture content of material exiting the press was in the range of 60-70%, frequently in the 60-65% range (Comparable moisture content for a screw press might be 67-72%). The throughput generally ranged from 0.2-0.5 tons per hour, with an average around 0.4 tons per hour. Specific energy requirement ranged from 4-6 horsepower-hours per ton, roughly half that of a screw press. Consistent good results were dependent on synchronizing both the feeder/packer and the rotating center drain with the rotation of the six rolls such that a uniform, solid plug of material was maintained in the press chamber.

Conclusions

The press configuration, which was evaluated, performed well in expressing juice from macerated alfalfa. Its specific energy requirement was lower than that of a screw press. It appeared to have the potential to be scaled up to industrial size, say 35-40 tons per hour throughput. If throughput is assumed to be proportional to chamber volume and the diameter/length ratio of the chamber remains constant, the press throughput would be proportional to the chamber diameter cubed. Scaling up from 0.4 tons/hour to 40 tons/hour would require a chamber diameter of approximately 30 inches and a length of 120 inches. Whether the scaled up press would function with exactly the same efficacy as the smaller version is open to conjecture.

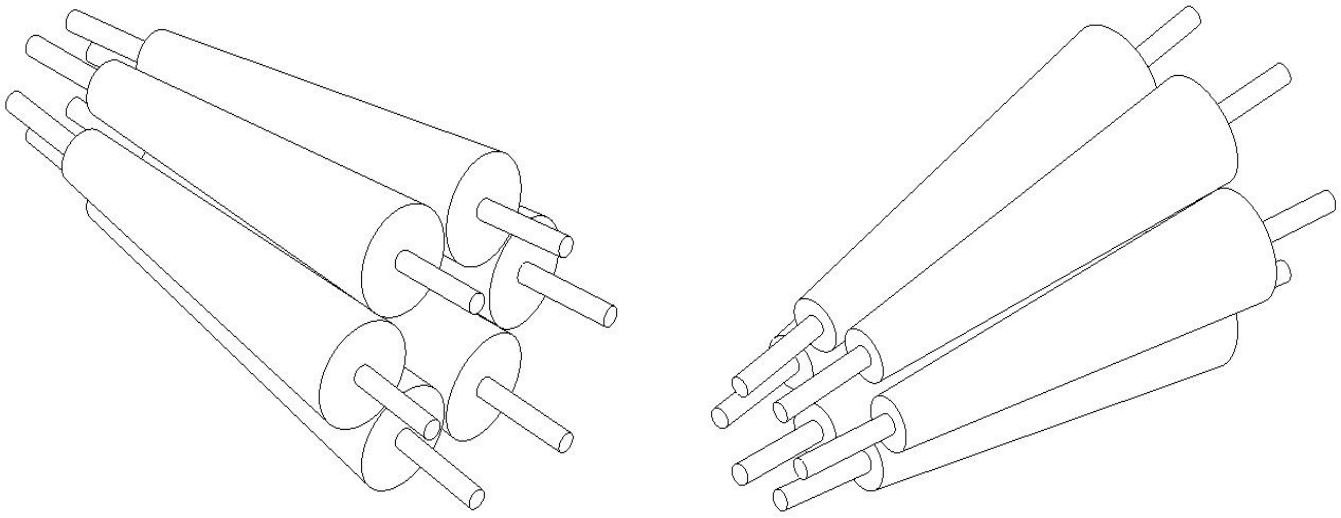

Figure 1. Rolling-compressing press.

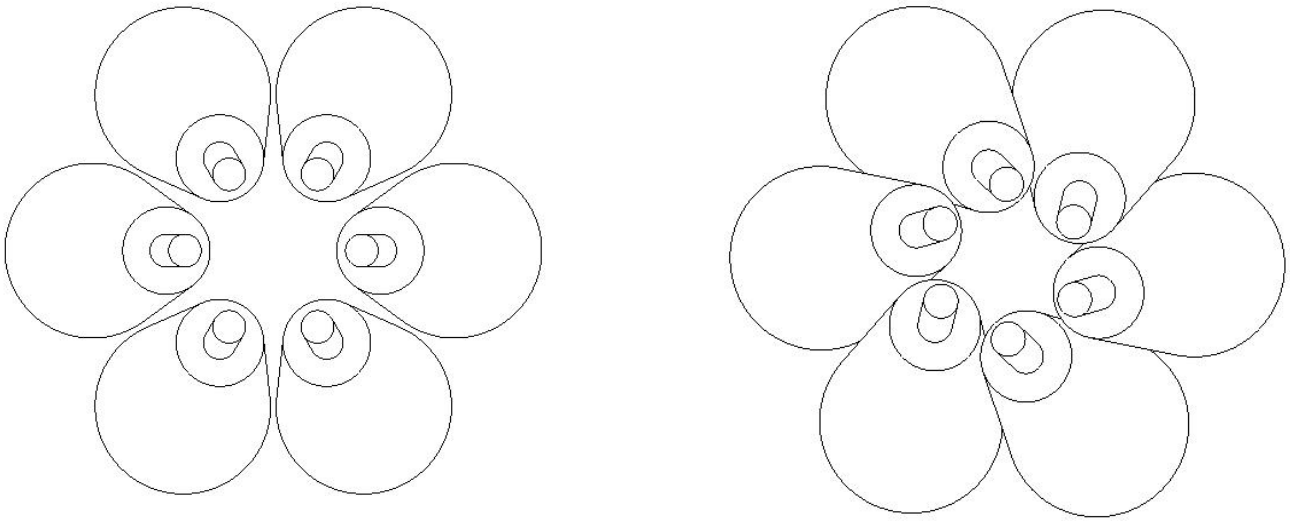

Figure 2. View showing rollers unskewed and skewed.

Inoculant Effects on Aerobic Stability of Corn Silage
R.E. Muck

Introduction

Inoculants are the most common additives used in making silage in the U.S. These products provide selected lactic acid bacteria to supplement the natural population of lactic acid bacteria on the crop and ensure a rapid and efficient silage fermentation. While these products have provided improvements in dry matter recovery and animal performance, aerobic stability (the time until the silage begins to heat during feed out) has sometimes been made worse by inoculants, particularly in corn and other whole-crop grain silages. Inoculant manufacturers are aware of this problem and have been working on developing inoculants that more consistently improve aerobic stability. The objective of this study was to test several new types of inoculants and compare their effects on aerobic stability with current products.

Methods

Whole-crop corn was harvested with a forage harvester without crop processing. The chopped corn was ensiled in 37 x 10 cm dia. PVC silos sealed with a rubber end cap on one end and with black plastic secured with duct tape on the other. There were eight treatments (an uninoculated control and seven inoculants) with four replicate silos per treatment. Three of the inoculants were standard corn silage inoculants available in the market, one was a new product with improved homofermentative strains, two products were with the heterofermentative species *Lactobacillus buchneri*, and the final product was a standard product with the addition of a chemical spoilage inhibitor. All products were applied at recommended rates but were diluted with water such that each treatment was applied at 1 g/50 g crop. The control received 1 g water/50 g crop. The silos were opened after 90 days of ensiling. Silos were weighed prior to emptying. The spoiled silage on the top was removed and weighed. The rest of the silage was removed, mixed and analyzed for microbial groups, pH, fermentation products and moisture content. The remainder was placed in Styrofoam buckets, and silage temperatures were recorded hourly until heating occurred.

Results and Discussion

The majority of analyses are complete, and key results are summarized in Table 1. The standard inoculants had no effect on the pH of the 90-d silages relative to the control; all had excellent pHs of 3.81 to 3.86. Of the new inoculants, only the *L. buchneri* inoculants had pHs significantly different from the control (3.90 and 4.01 vs. 3.82). The standard inoculants and the improved standard inoculant generally had the numerically lowest gaseous losses and gaseous plus spoilage losses on a wet weight basis although trends were not quite statistically significant.

Of the four improved inoculants, three produced significant improvements in aerobic stability. The *L. buchneri* inoculants and inoculant plus chemical spoilage inhibitor had aerobic stabilities more than double that of the uninoculated control. Only the inoculant with improved homofermentative strains failed to provide a benefit. Aerobic stability across the treatments was negatively correlated with yeast counts as indicated in Fig. 1. These results suggest that the primary means of improving aerobic stability in the effective products were by lowering yeast populations, the frequent initiators of heating in corn silage.

One trial, particularly with laboratory-scale silos, is insufficient to draw general conclusions. First, acetic acid bacteria are often initiators of aerobic deterioration in farm-scale corn silage. These bacteria were at similar or higher populations than the yeasts in this trial but did not appear to be factors in the current trial because their counts were similar across all treatments. Some of the new products may be more or less effective if the acetic acid bacteria initiated spoilage. Second, multiple trials are needed to establish a true pattern of activity. Finally, animal trials will be important to establish whether the new inoculants perform similarly to standard products. Animal performance improvements are the primary means by which farmers receive a direct return for using these products. So any changes in animal performance will be important in determining the economic benefits of the new inoculants.

Conclusions

In this preliminary trial, two new types of silage inoculants (*L. buchneri* and a standard inoculant with a chemical spoilage inhibitor) doubled the aerobic stability of corn silage compared to that of an uninoculated silage under conditions where yeasts appeared to be the initiators of aerobic deterioration. Additional studies are needed to confirm the value of these approaches.

Table 1. Characteristics of the 90-day silages.

Inoculant	pH	Aerobic Stability, h	Gaseous Loss % wet weight	Gaseous plus Spoilage Losses % wet weight
None	3.82	75	2.8	27.3
Standard 1	3.84	71	1.9	21.3
Standard 2	3.83	50	1.8	19.9
Standard 3	3.85	91	2.4	25.5
Improved Standard	3.81	51	2.0	20.4
Standard + Inhibitor	3.83	152	2.6	27.1
L. buchneri 1	4.01	217	2.4	23.3
L. buchneri 2	3.90	178	2.3	26.8

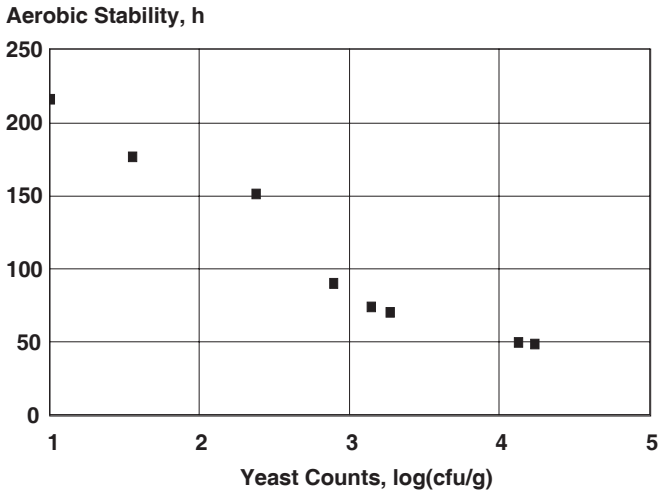

Figure 1. Aerobic stability of the treated silages as correlated with yeast counts in the silages at opening. A yeast count of 1 log (colony-forming units/g crop) indicates that all four silages in the treatment were below detectable level (100 cfu/g crop).

The Cost of Unloading Silos Immediately

R.E. Muck

Introduction

Particularly on small farms with one tower silo per crop, it is common to find "silage" being removed daily from silos during the filling process. This "silage" is often unfermented or poorly fermented, may heat rapidly in the feed bunk, and may result in poor animal intake and performance. While such practices are not recommended, little is known about the true cost of such practices. This study was initiated to look at the consequences within the first two weeks of ensiling.

Methods

Seven similar trials were performed over two years, five with alfalfa (26, 35, 36, 47, 65% DM) and two with whole-crop corn (31, 38% DM). Forage was harvested with normal field equipment and ensiled in 1.5 m x 15 cm dia. PVC pipe silos. Dry matter densities ranged from 145 to 215 kg/m^3, representing typical densities that might be expected in a leveled, packed by foot upper layer of a tower silo. The silos were stored with 10-cm thick fiberglass insulation around the walls. There were four treatments (covered with plastic, uncovered, 5 cm/day removed, 10 cm/day removed) with two silos per treatment. Each trial lasted 14 d. For the two treatments with forage removed daily, the amount removed was weighed and analyzed for pH, moisture, ash, fermentation products, crude protein and various N fractions. Enumeration of microbial groups was performed on all days except for weekends. At the end of 14 d, remaining silage in all treatments were analyzed similarly by depth (0-10, 10-20, 20-30, 30+ cm from top). Temperatures by depth were recorded daily for all treatments.

Results and Discussion

The pH profiles across treatments generally followed a pattern similar to those shown in Fig. 1. The profiles in the covered and uncovered treatments are the pHs at day 14 whereas the last four and two points for the 5 and 10 cm/d treatments, respectively, are from day 14, the rest being the pHs of daily samples. In the covered treatment, pH was elevated in the top 10 cm relative to samples from deeper depths indicating some air ingress under the plastic. In the uncovered treatment, spoilage was evident by elevated pHs in the top 20 cm, but deeper samples were of uniform pH. In treatments with daily removal, pHs were elevated for the first few days but by 7 d the pHs were only several tenths of a pH unit above those at the same depth in the undisturbed treatments. By 10 to 14 d, the pHs in the daily removal treatments were similar to those in the undisturbed treatments. The only exception to this pattern occurred in the 65% DM alfalfa silage. In this trial, the pHs of the daily removed forage were above 6.0 throughout the trial. Only on day 14 when the remainder was emptied were pHs of similar value to those in the undisturbed treatments found.

Silo temperature profiles (5, 10, 15, 20 and 40 cm below the surface) in the daily removal treatments were higher than those in the covered treatment for between 5 to 12 d. Thereafter temperatures across the three treatments were similar. The only exception was the 65% DM alfalfa silage where temperatures were elevated throughout the trial in the daily removal treatments. Across all trials, the highest sustained temperatures were in the uncovered treatment where temperatures in the upper 20 cm were continually above ambient throughout 14 d.

The temperature and pH data together suggest that relatively stable silage was reached in the daily removal treatments within 7 to 14 days with the exception of the driest alfalfa trial. However, microbial profiles suggest that the silages from the daily removal treatments may not be particularly stable upon reaching the feed bunk. In corn silage, yeast counts in the day 14 samples from the daily removal treatments were 10^6 to 10^8/g crop, 10 to 100 times higher than those found in the majority of silage from undisturbed treatments (samples below 20 cm from the top). Silages with yeast counts above 10^5/g are considered susceptible to heating in the feed bunk. Consequently none of the corn silage treatments would have been considered stable, but the daily removal treatments would have been rated as highly susceptible to heating. Similar results were observed in the 47 and 65% DM alfalfa silages. These high DM alfalfa silages also had elevated levels of enterobacteria. Yeast counts in the alfalfa silage of less than 40% DM were at or below detectable level by day 14 in all treatments with the exception of the top 20 cm of the undisturbed treatments. All of the 14-d silages in these three trials would have been expected to be aerobically stable.

Conclusions

Even when removing forage from a silo on a daily basis immediately after filling, evidence of fermentation in the removed feed appeared rapidly (< 7 days) with the exception of a very dry forage (65% DM). Silo temperatures also suggest a stabilization within 14 days. However in corn silage and drier alfalfa silage (> 45% DM), yeast levels were still elevated at 14 days, suggesting an aerobically unstable feed supply for an even longer period. These results indicate the importance of sealing the silo and allowing the crop to ferment and stabilize properly. With alfalfa, our results suggest that filling the top of the silo with wetter forage (35 to 40% DM) can reduce the problems of immediately feeding from a top-unloading tower silo.

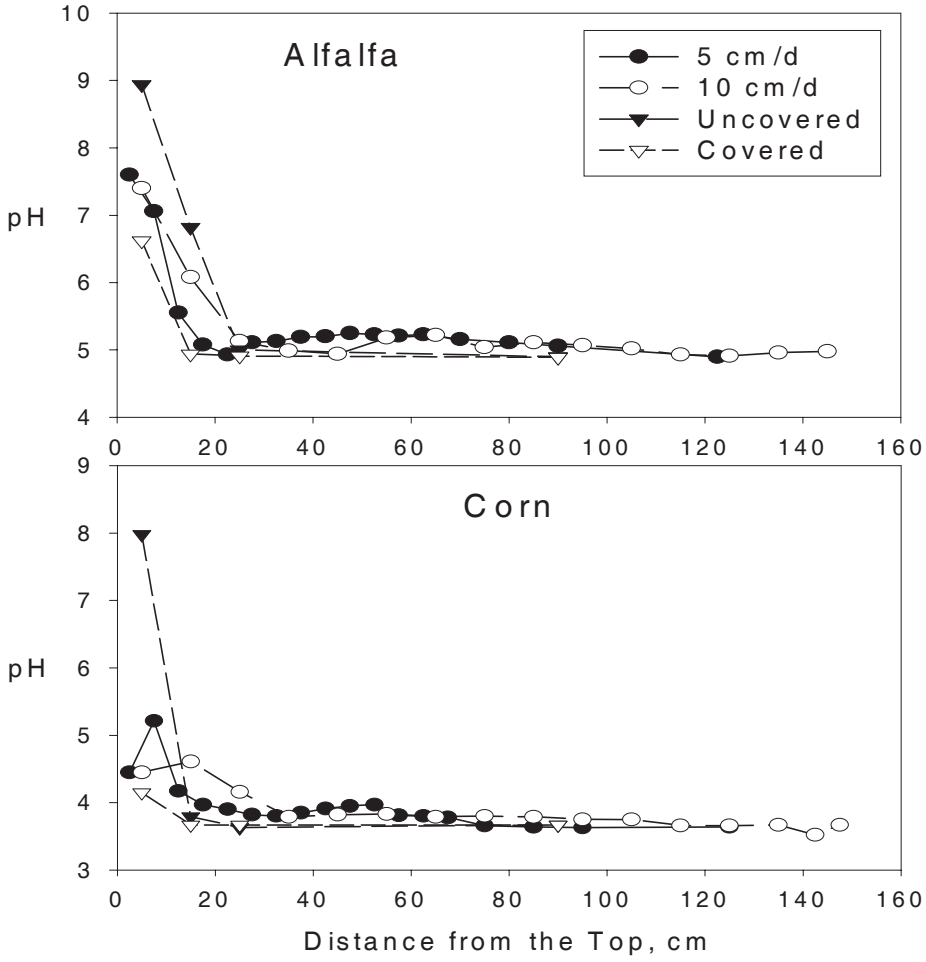

Figure 1. Silage pH profiles in representative alfalfa (36% DM) and corn (31% DM) silage trials as influenced by treatment.

Unique Lignin Dimers from a CAD-deficient Mutant

J. Ralph, J. Peng, and F. Lu

Introduction

Lignification produces the complex lignin polymer in the plant cell wall that is vital for structural integrity of land-based plants, for defense against pathogens, and to facilitate various functions such as water transport. Lignin is unusual compared to other abundant natural polymers (such as cellulose and proteins) due to the low degree of order and the high degree of heterogeneity in its structure. We have recently been interested in understanding how plants respond to deviations in the normal lignification process. Some such deviations occur as natural mutants; others can be developed using genetic engineering approaches. The potential to improve plant utilization by ruminants and in various other natural and industrial processes by engineering the amount, composition, and structure of lignin is currently attracting considerable interest.

The "DFRC" method (Derivatization Followed by Reductive Cleavage) developed at the Dairy Forage Research Center provides many useful analyses of lignin. In this paper, the dimer fingerprint region is utilized to illustrate the striking differences between lignins in a normal plant and in a CAD-deficient mutant.

Results and Discussion

Although hardly a forage, the availability of a pine CAD-deficient mutant allows us insight into the basic processes associated with lignification.

Figure 1 shows GC-MS of the DFRC dimers from the mutant vs a normal pine. In the normal lignin, we can identify and recognize anticipated dimers, notably the β-1-, β-β-, and β-5-dimers, from a softwood lignin. These dimers are present in small amounts in the mutant's product, but are overwhelmed by a series of new dimers. . From the fingerprint alone, it is obvious that these two lignins must differ greatly.

The new dimers turn out to be the various coupling products between vanillin (**V**) and dihydroconiferyl alcohol (DHCA, **D**), which have been shown previously to be substantially elevated in the mutant's lignin. Since these dimers are released from the polymer by the DFRC process, they must be attached as β-ethers and are therefore involved in cross-linking with normal lignin monomers. The same products in similar distributions arise when whole-cell-wall samples, rather than simply the isolated lignins, are subjected to DFRC.

Conclusions

The DFRC dimers provide a sensitive fingerprint for detecting anomalies in lignins. In the CAD-deficient mutant examined, overwhelmingly major products arise from coupling of compounds not traditionally associated with lignification, aldehydes and DHCA. After further refinement, this method will be used to examine lignins in agriculturally important crops, in attempts to improve the digestibility of forage cell wall polysaccharides.

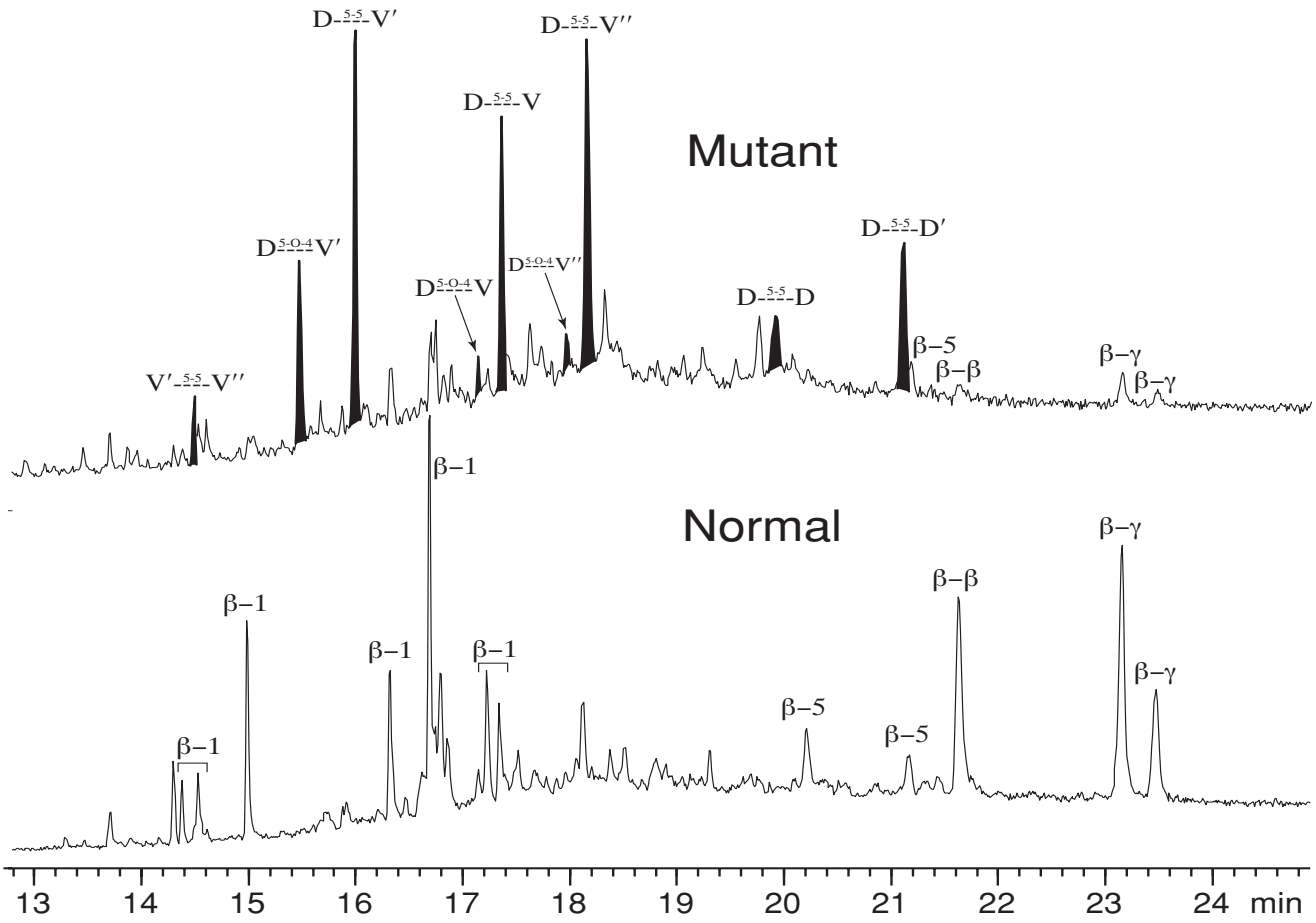

Figure 1. DFRC dimers from normal vs. mutant pine lignins. Although the normal dimers can be detected in the CAD-deficient mutant, they are overwhelmed by new products that are insignificant in normal pine. These new dimers are all various combinations of DHCA (D) (or its derived guaiacylpropane-1,3-diol (D′)) and vanillin (V) or its DFRC-derived products (V', V"). The bonding patterns are shown in the peak labels. The same products appear in similar distributions whether the DFRC-degradation is performed on the isolated lignins or whole-cell-wall materials. From the fingerprint alone, it is obvious that the two lignins are significantly different.

Incorporation of Aldehydes into Lignins

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Introduction

Aldehydes are well-known components in lignins and are responsible for the characteristic phloroglucinol staining of lignified tissues. Whether they are true components of lignin from co-polymerization (radical cross-coupling) with monolignols/oligolignols has recently become important to elucidate as the lignins from various CAD-deficient plants are examined.

Cinnamyl alcohol dehydrogenase (CAD) catalyses the final biosynthetic step from coniferaldehyde to coniferyl alcohol, and from sinapaldehyde to sinapyl alcohol, the primary components of lignins. Down regulation of CAD in a variety of mutants and transgenics leads to an accumulation of hydroxycinnamaldehydes (at the apparent expense of the monolignols) and an apparent buildup of their content in resultant lignins.

It has long been recognized that coniferaldehyde is a viable substrate for free-radical coupling reactions analogous to those that occur with the standard monolignols. It is possible to make coniferaldehyde synthetic lignins (DHPs) for example (Higuchi *et al*., 1994). Here we examine dimerization and oligomerization of coniferaldehyde, and copolymerization of coniferaldehyde with coniferyl alcohol to produce synthetic lignins. Spectral data from these model reactions are used to provide a more substantive elucidation of the structures of aldehyde moieties in lignins, particularly those from mutant and transgenic plants that are CAD-deficient.

Experimental

Dimers, mixed oligomers, and synthetic lignins were made using peroxidase and H_2O_2 by rather standard methods that will not be detailed here. The mutant pine and transgenic tobacco lignins were isolated (by dioxane-water extraction of ball-milled cell wall material) as has been described (Ralph *et al*., 1997; Ralph *et al*., 1998). More details is available in the full conference paper (Ralph *et al*., 1999a) which is available on our website, http://www/dfrc/ars/usda/gov under Full-text Publications.

Results and Discussion

Model Reactions. Radical coupling of coniferaldehyde using peroxidase/H_2O_2 produced mixtures from which the parent dimers of structures **1**-**4**, Fig. 1, could be isolated and characterized by NMR, Fig. 2. Most of the products have been described previously, but without sufficient NMR data for the current study. Useful insight into the relative amounts and the nature of the dimers was gained from small-scale (5-10 mg) experiments using [9-^{13}C]coniferaldehyde. Assignments could be readily made from 1D ^{13}C-NMR of the mixture which, because of the [9–^{13}C]-labeling, could be acquired in minutes, and from 2D HSQC-TOCSY and HMBC experiments where the aldehyde carbon region could be selectively and quickly acquired. Analysis of the crude dimer/oligomer mixtures revealed an unexpected product.

When coniferaldehyde dimerizes with one of the radicals coupling at its 8-position, the resulting intermediate product is a quinone methide; this is analogous to the dimerization of coniferyl alcohol with one of the radicals coupling at its β-position. In the case of the aldehydes, however, the quinone methide has new options. The resultant 8-proton is particularly acidic because of the aldehyde group; elimination of the 8-proton allows re-aromatization. Thus, the 8–O–4-dimer produced is **1**, Fig. 1 — the addition of water to the quinone methide cannot compete with the faster 8-proton elimination. An 8–O–4/8–O–4 trimer was also isolated. The 8–8-dimer **3** similarly regains 7,8-unsaturation by 8-proton elimination from the di-quinone methide coupling intermediate. The products are both analogous to those produced by ferulate where the intermediate quinone methides also have acidic (but less so) 8-protons (Ralph *et al*., 1994). The surprise was that the 8–5-product was the cyclic phenylcoumaran structure **2c**. Although this product is analogous to that formed from coniferyl alcohol or ferulate, it had been thought that 8-proton elimination was faster than intermolecular trapping of the 8–5-quinone methide intermediate. Indeed the previously reported product was the opened structure **2o** (Connors *et al*., 1970). Attempted isolation of **2c** from the mixture produced only the ring-opened elimination product, dimer **2o**. Acetylation of the mixture also converts the phenylcoumaran **2c** to the (acetate of) acyclic dimer **2o**, as shown in Fig. 2. Proof of the structural assignment for the phenylcoumaran 8–5-product **2c** is shown in Fig. 3, an HMBC experiment which also validates the other assignments (see caption).

In synthetic cross-coupling reactions, as seen in the DHP example, Fig. 2, that copolymerizes [9–^{13}C]-coniferaldehyde (10%) with coniferyl alcohol (90%), one new product arises. The peak labeled **3'** remains unauthenticated at present but is thought to be the 8-β'-cross-product **3'**. If so, its minor change in chemical shift indicates that, like the 8–8-coupled dimer **3**, the aldehyde moiety remains unsaturated. This means that the intermediate quinone methide produced following radical coupling was not efficiently trapped internally by the γ'–OH. Thus, unlike the 8–5-coupling product, 8-proton elimination is faster here than internal trapping. The copolymer synthetic lignin also contains 8–O–4-cross-coupled aldehyde units **1*** are **1'** that were not seen in the coniferaldehyde-only reactions. One other feature of the synthetic lignification is notable: most of the coniferaldehyde appears *not* to have coupled at its 8-position.

The dominant **V** peak indicates that any cross-coupling entered into by coniferaldehyde was predominantly at the ring 5- or 4–O-positions. As will be discussed below, this is not reflected in the isolated lignins. The reason likely results from the considerably more "bulk" nature of synthetic lignification. With excess coniferyl alcohol in the system, the likely coupling reaction is between coniferaldehyde and coniferyl alcohol monomer radicals. As has been seen countless time, coniferyl alcohol overwhelming couples at its β-position in cross-coupling reactions. Although the aldehyde may also couple at its 8-position, as seen from the (unauthenticated) **3'** peak, it more commonly couples at the 4–O- or 5-position, as evidenced by the large **V** peak.

Due to severe matrix and solvent-dependent shifts, the model data (in acetone-d_6) and the lignin spectra do not appear to coincide — see caption to Fig. 2. The direction of the shifts upon adding water (necessary for the solution of unacetylated lignins) is illustrated with the crude dimer products and the copolymer DHP. Dotted assignment lines on Fig. 2 have been authenticated by further 2D correlation experiments (not shown). Once the models and the lignins are acetylated, the data coincide much more closely (right-hand plots in Fig. 2).

Lignin Spectra. The aldehyde-carbon region of spectra from lignin isolates are also shown in Fig. 2, where the caption describes details of the assignments and findings. In the CAD-deficient pine mutant, the typical cinnamaldehyde **V** and benzaldehyde **U** peaks are present at enhanced levels, consistent with the build-up of coniferaldehyde in a plant deficient in CAD. More important are the peaks from 186-189 ppm which are due to 8–O–4-coupling and cross-coupling products **1**. {These new aldehyde components were misidentified originally (Ralph et al., 1997), but corrected in subsequent publications (Ralph et al., 1998; Ralph *et al*., 1999b). (A book chapter on NMR of Lignins (Ralph et al., 1999b) even details how the incorrect assignment was made).}

In the CAD-downregulated tobacco transgenic, the isolated lignin contains striking aldehyde components. 8–O–4-products **1*** and **1'** abound and other NMR experiments determine that they are largely sinapaldehyde-derived in this case (tobacco produces syringyl-guaiacyl lignins) (Ralph et al., 1998). Presumably due to the large aldehyde concentrations, 8–8-coupled products **3** are also seen. Sinapaldehyde, like sinapyl alcohol, favors 8–8-coupling, in part because there are fewer options than for coniferaldehyde (which has a 5-position available for radical coupling).

Implications. The isolated phenolic polymers from the mutant pine and the transgenic tobacco contain significant aldehyde components, copolymerized in by radical coupling reactions that typify lignification. The polymers have significant molecular weight (~13 kDa for the pine isolate) and contain cross-coupling products of coniferaldehyde/sinapaldehyde with lignin oligomers (e.g. 8–O–4'-structures **1*** and **1'**) as homo-coupling products such as **3**. They therefore appear to be true components that are polymerized by radical coupling mechanisms into a phenylpropanoid polymer that may function as lignin. This is not the same polymer that would be produced (and can be isolated) when the plant does not have a CAD-deficiency. It contains significantly more aldehydes (as well as another major component, dihydroconiferyl alcohol units, in the pine mutant) (Ralph et al., 1997; Dimmel *et al*., 1999). It appears, as originally proposed (Ralph et al., 1997), that the plants are utilizing these hydroxycinnamaldehyde precursors of the normal monolignols when the plant is unable to provide sufficient quantities of the monolignols for normal lignification. Hydroxycinnamaldehydes are logically anticipated to build up if the flux through the final reduction step, catalyzed by CAD, is reduced. The total levels of the phenylpropanoid polymers are close to those in the normal plants (Ralph et al., 1997; MacKay *et al*., 1997; Yahiaoui *et al*., 1998; Ralph et al., 1998). However, it appears from the build-up of extractable hydroxycinnamaldehyde and benzaldehyde monomers in the plant stems that producing the polymer from these components is not straightforward. In synthetic systems too, coniferyl aldehyde does not appear to be as readily incorporated as the alcohols. Nevertheless, the hydroxycinnamaldehydes, and their derived hydroxybenzaldehydes, become a significant part of the polymer fraction.

There has been considerable debate about whether these phenylpropanoid polymers are in fact lignin, and whether they are functioning as lignin in the plant. A great deal remains to be resolved. Aldehydes have always been considered to be associated with lignins (Chen, 1992). Apart from their obvious presence ascertained from phloroglucinol staining, we see them in isolated lignins by NMR and other spectroscopies, and they are clearly present in intact cell walls of plants as seen by the release of characteristic products from the many degradative methods. Could they be artifacts? Indeed, but they are certainly in the polymers we think of as lignins in normal plants. Finding higher levels in CAD-deficient mutants and transgenics seems logical.

The important question to be answered from the observations reported here, however, is: are aldehydes incorporated, as monomers, into lignins or are they simply post-lignification artifacts of oxidation? Peroxidase/$H_2O_2$is capable of producing aldehyde monomers from monolignols, so they may be expected in the lignifying zone even in the absence of CAD-deficiencies. The high levels on aldehydes in lignins from CAD-deficient mutants and transgenics and their incorporation of monomers by homo- and hetero-coupling reactions into polymeric fractions makes it clear that they can indeed enter into the phenylpropanoid polymer fractions by the mechanisms characteristic of lignification. For most researchers, there is little surprise in this. If phenols are present in the cell wall during lignification, and if lignification is not carefully enzymatically controlled (Ralph *et al*., 1999c), it is

logical that they will incorporate into the polymer, depending only on their abilities to form radicals and their cross-coupling propensities under the conditions of lignification. Many other non-monolignols (e.g. ferulates, acylated monolignols) have been shown to be components of lignins, so there is ample precedence for the incorporation of non-monolignols into lignins. Further studies are required to determine if the aldehyde-rich polymers function as lignins. However, it should be noted that, if these phenolic polymers (containing significant levels of aldehydes that have clearly incorporated hydroxycinnamaldehyde monomers by radical coupling reactions) are *not* to be classified as lignins, then these CAD-deficient mutant and transgenic plants are surviving nicely with very little lignin!!

Conclusions

Hydroxycinnamaldehyde monomers appear to incorporate well into synthetic and natural lignins as anticipated from the currently accepted lignification mechanism. The radicals couple in a variety of anticipated ways. Lignins isolated from plants with deficiencies in the enzyme CAD have elevated levels of aldehydes with bonding patterns discernable from NMR that provide evidence suggesting the incorporation of monomeric aldehydes (8–O–4-cross-coupled structures). Work is still required to determine whether the plant is truly producing a modified lignin by incorporating the aldehyde monolignol precursors to ensure the viability of the plant, or produces this polymer as some kind of a wound response. Either way, if such transgenic plants are to be utilized as forages for ruminants, or for chemical pulping, these polymers contribute to the non-cellulosic portion and it is logical to classify them broadly as lignins. Processing problems or advantages of such modified plants will be interesting to follow in the near future.

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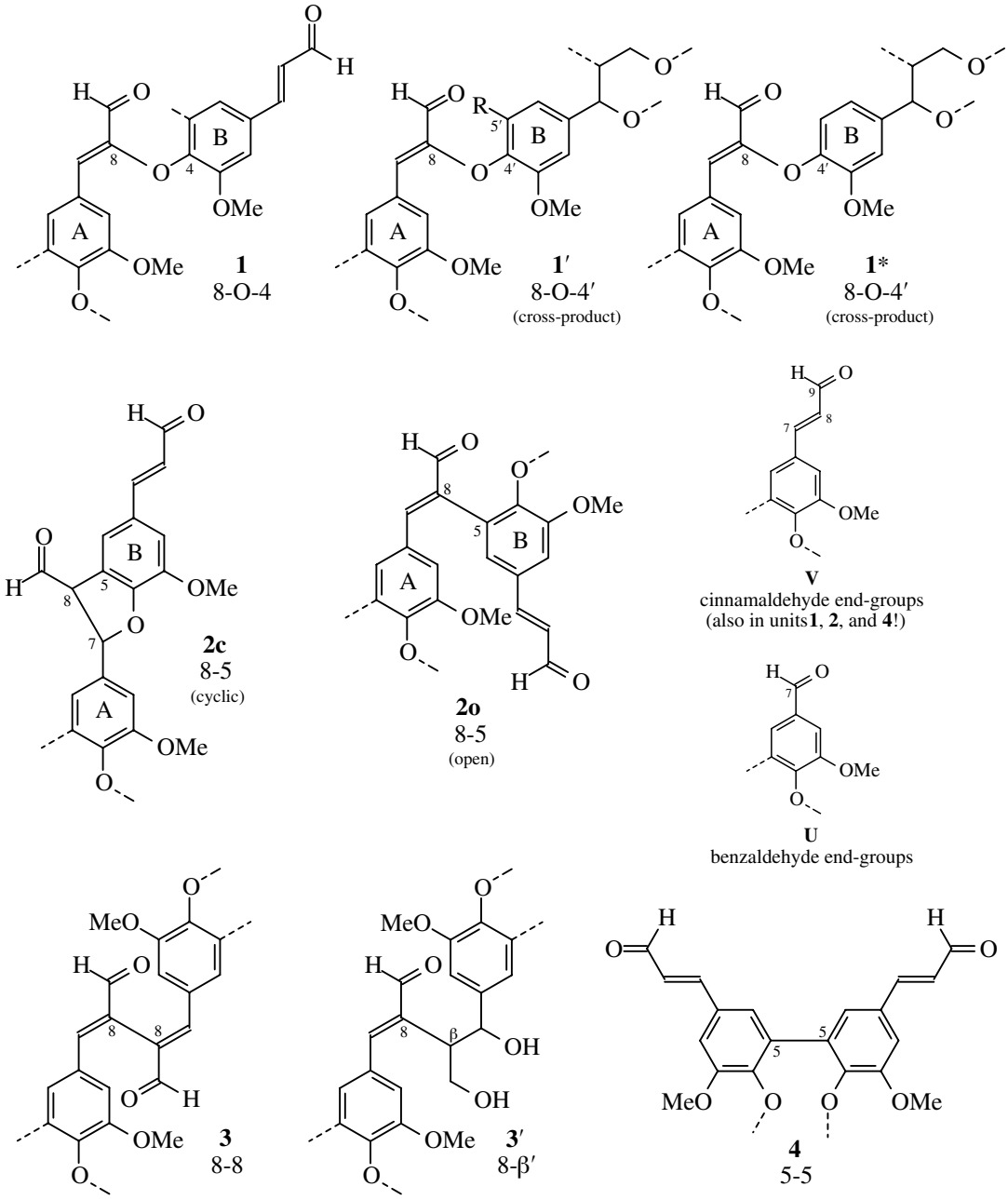

Figure 1. Structures of various units derived from radical coupling of hydroxycinnamaldehydes. Structures **1**-**4** result from homo-coupling of hydroxycinnamaldehydes. Units **1'** are from cross-coupling of a hydroxycinnamaldehyde radical with a radical from a preformed 5-substituted (e.g. syringyl) lignin oligomer (B-moiety). **1*** is from (cross-) coupling with a guaiacyl moiety. Hydroxycinnamaldehyde endgroups **V** can arise from incorporation of a hydroxycinnamaldehyde monomer into lignin, or remain (as the B-moieties) following homo-coupling of hydroxycinnamaldehydes (in structures **1**, **2**, and **4**, for example). Hydroxybenzaldehyde units (producing benzaldehyde endgroups **U**) result from hydroxycinnamaldehydes. It is not known whether they are result from direct incorporation of hydroxybenzaldehyde monomers (vanillin and syringaldehyde) into lignin, or are produced post-lignification from hydroxycinnamaldehyde **V** units in lignin; the former is strongly implicated in the current work, but is not necessarily exclusive. It is not yet known whether units **4** exist as such in lignins — the dimer can clearly form, but radical coupling is still possible at the 8- and 4–O-positions; various types of 5–5-coupled products (including dibenzodioxocins) are possible. Finally, note that cross-coupling products other than **1'** are possible and are presumably responsible for the broadening/multiplicity seen in some of the ^{13}C-NMR peaks in the lignin spectra of Figure 2.

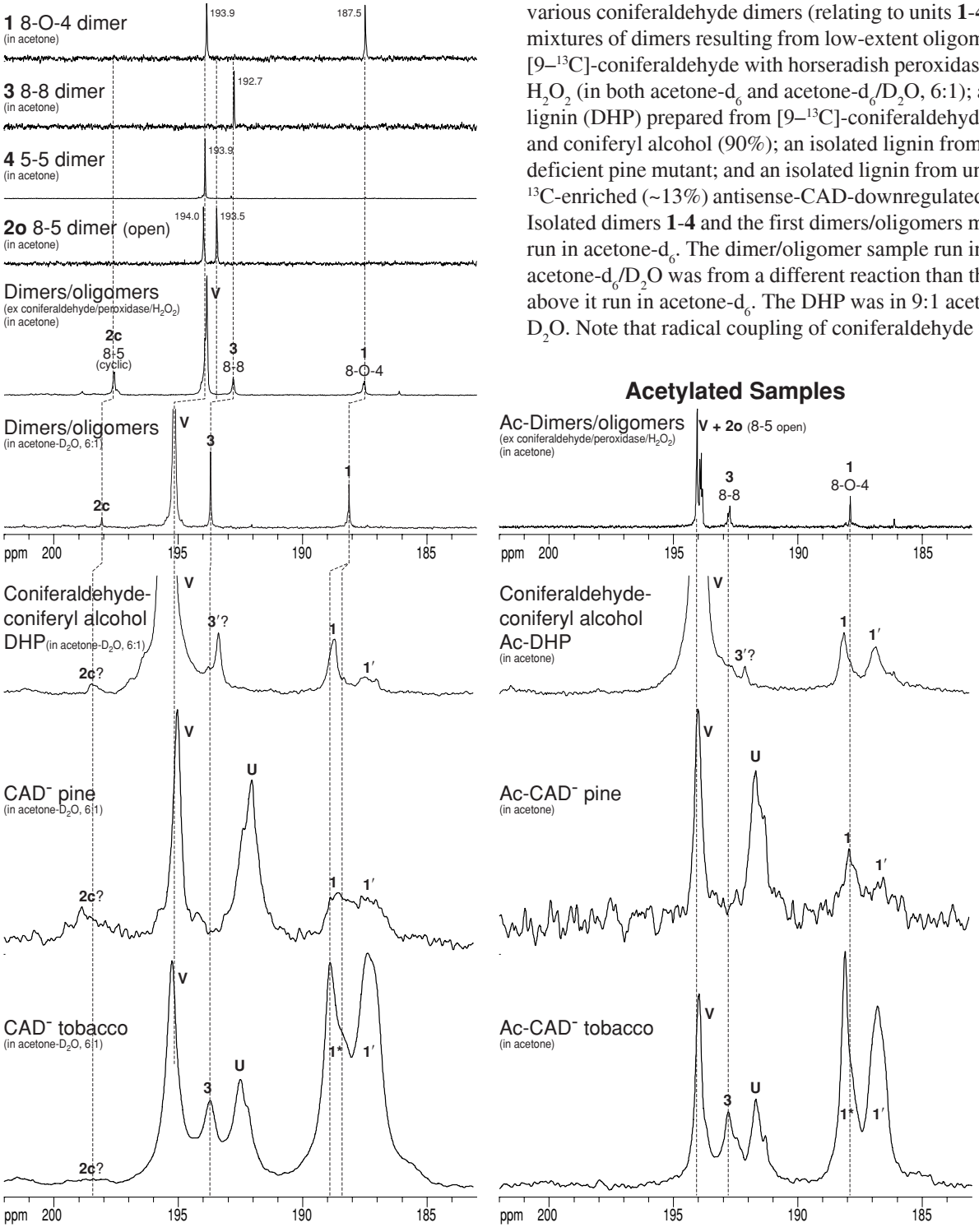

Figure 2. Left: aldehyde sub-regions of ^{13}C-NMR spectra of: various coniferaldehyde dimers (relating to units **1**-**4**); crude mixtures of dimers resulting from low-extent oligomerization of [9–^{13}C]-coniferaldehyde with horseradish peroxidase and H_2O_2 (in both acetone-d_6 and acetone-d_6/D_2O, 6:1); a synthetic lignin (DHP) prepared from [9–^{13}C]-coniferaldehyde (10%) and coniferyl alcohol (90%); an isolated lignin from a CAD-deficient pine mutant; and an isolated lignin from uniformly ^{13}C-enriched (~13%) antisense-CAD-downregulated tobacco. Isolated dimers **1**-**4** and the first dimers/oligomers mixture were run in acetone-d_6. The dimer/oligomer sample run in 6:1 acetone-d_6/D_2O was from a different reaction than the one above it run in acetone-d_6. The DHP was in 9:1 acetone-d_6/D_2O. Note that radical coupling of coniferaldehyde via peroxidase/H_2O_2 clearly produces the cyclic (phenylcoumaran) 8–5-dimer corresponding to structure **2c**, as proven in Fig. 4; we have not yet been able to purify dimer **2c** — it ring-opens and eliminates (see Fig. 3) to the acyclic (open) form **2o** (for which we do have the dimeric model). We do not currently have a model compound for the 8–β-cross-product **3'** seen in the copolymer DHP, so this peak remains unauthenticated. If it is the 8–β'-cross-product, it appears to be in the open form as shown in Fig. 1, in which the intermediate quinone methide on the aldehyde moiety is not internally trapped by the γ–OH (as occurs in β–β-coupling of coniferyl alcohol to pinoresinol). Substantial solvent and matrix-dependent shifts are noted for the unacetylated dimers, oligomers, and the polymers (which were run in ~6:1 acetone-d_6/D_2O). The assignment lines linking the various peaks in the two lignin spectra have been authenticated by further correlation experiments (not shown) on the lignin

samples. The CAD-deficient pine lignin shows the typical benzaldehyde **U** and cinnamaldehyde **V** peaks (although significantly enhanced over the corresponding lignin from normal pine), along with evidence for 8–O–4-coupled coniferaldehyde units **1*** and **1'**. The CAD-downregulated tobacco has significant levels of hydroxycinnamaldehyde (mainly sinapaldehyde) incorporation producing high levels of 8–O–4-coupled products **1*** and **1'**; the 8–8-coupled product **3** is also clearly evident. Right: Corresponding spectra of acetylated oligomeric products and lignin isolates. Solvent shifts are not a problem in this case since all samples dissolved in acetone-d_6. Thus the assignments of 8–O–4- and 8–8-coupled products are verified (and further authenticated by diagnostic correlations in various 2D NMR experiments). Note that the cyclic phenylcoumaran form of the 8–5-dimer **2c** converts to the opened product **2o** upon acetylation . The assignment of peaks at ~186.5 ppm (and 187 ppm in the unacetylated lignins) as cross-coupling products **1'** is from other NMR data (not shown) — we hope to have model data for this before long.

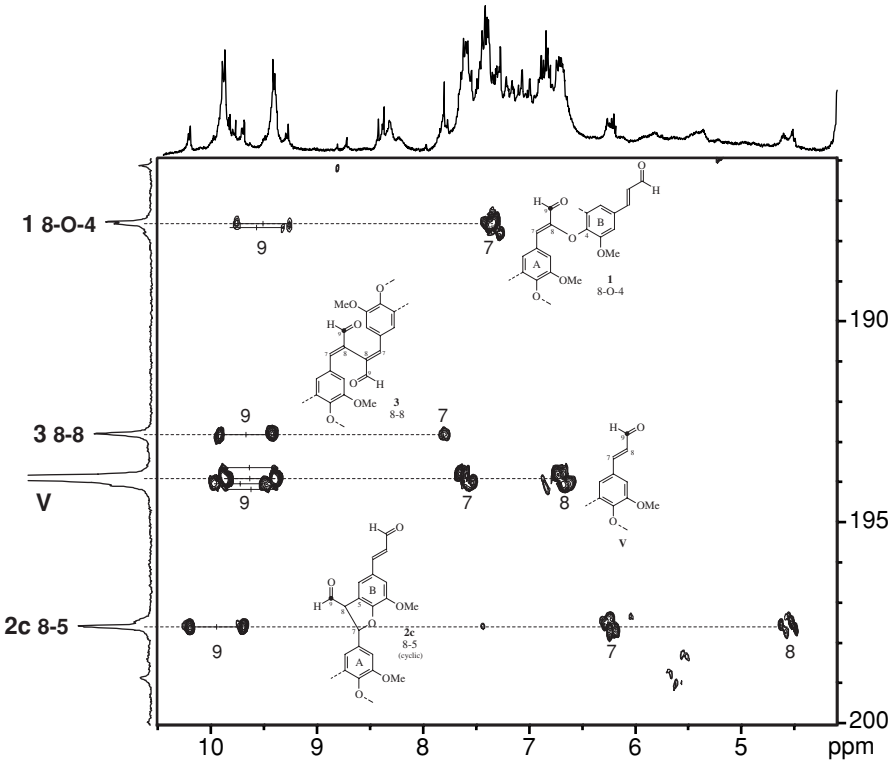

Figure 3. NMR evidence for the dimer/oligomer assignments of aldehyde ^{13}C-NMR peaks to structures **1**-**4** and **V**, and proof that the 8–5-dimer is in the cyclic phenylcoumaran form following peroxidase-mediated radical coupling at around neutral pH. The ^{13}C—^{1}H long-range correlation experiment (gradient-edited HMBC, solvent: acetone-d_6) correlates each aldehyde carbonyl carbon with its directly attached aldehyde proton (split by the 1-bond ^{13}C—^{1}H coupling constant — the proton chemical shift is halfway between the pair of correlation peaks) and other protons 2- or 3-bonds away (in this case, protons 7 and 8 on the sidechain). The 8–O–4- **1** and 8–8-units **3** are clearly unsaturated as shown by their correlations to single 7-protons. End-units **V** (which also comprises the B-moieties of units **1**, **2**, and **4**) show correlations to both unsaturated sidechain protons (7 and 8). The interesting 8–5-unit **2c** clearly shows its phenylcoumaran nature — the ring-opened elimination product **2o** has only one proton correlation (its aldehyde carbon and correlated 7-proton coinciding with those of units **V**. Clearly, therefore, internal trapping of the intermediate quinone methide product of radical coupling is faster than H–8 proton elimination (analogous to that seen following 8–O–4- and 8–8-coupling). The 8–5-coupling product has always been reported as its opened form **2o**; there is weak evidence (Fig. 2) that (unacetylated) lignins contain some of the cyclic unit **2c**.

Arylpropane-1,3-diols in Lignins

J. Ralph, H. Kim, J. Peng, J. Scott, R. Sederoff, J. McKay, and F. Lu

Introduction

The hydroxyphenylpropanoid polymeric component of plants, lignin, can be dramatically altered when lignin-biosynthetic-pathway enzymes are downregulated in mutant or transgenic plants. Examination of the plant responses to such mutations gives us considerable insight into normal lignification and may reveal novel approaches to improving cell wall digestibility in ruminants. We recently characterized a pine mutant deficient in cinnamyl alcohol dehydrogenase (CAD) the enzyme catalyzing the conversion of 4-hydroxycinnamaldehdes to 4-hydroxycinnamyl alcohols, the monomers principally used in lignification. During lignification, these monomers are converted to radicals where they undergo radical coupling reactions with radicals from other monomers or, more commonly, from the growing lignin oligomer/polymer to build up a complex macromolecular structure. Because of the various possible coupling sites on these radicals, the resulting polymer is complex and contains a variety of interunit linkages.

Several mutants and transgenics in which the CAD level is downregulated incorporate the precursor aldehydes of the normal monolignols into their lignins. Thus in a CAD-deficient tobacco transgenic, sinapaldehyde becomes a major component of the lignin, radically coupling with other such aldehyde units and cross-coupling with traditional lignin components. Similarly, in the CAD-deficient mutant pine, coniferaldehyde units become significant in the polymer. But the more striking observation in the pine was the clear incorporation of significant quantities of an unexpected unit, dihydro-coniferyl alcohol (DHCA **2**), into the lignin. DHCA is found at lower levels in normal plant lignins but its derivation is not completely clear. Despite claims that the unit can only arise as a modified metabolic product *following* dimerization of traditional lignin monomers, we have presented ample evidence that it is formed as a monomer and that the monomer is incorporated into the lignin via radical coupling processes. Additionally, DHCA monomer is found in solvent extracts from these plants. It is however still not known whether it derives from coniferyl alcohol or can come via other pathways from coniferaldehyde. Since all indications in the pine mutant are that coniferaldehyde builds up and is not efficiently reduced to the alcohol, the significant DHCA component was conjectured to arise from coniferaldehyde rather than coniferyl alcohol, but this has not yet been elucidated.

Here we identify another significant component of the mutant pine's lignin, arylpropane-1,3-diols (APDs **1**), whose source was originally puzzling. It is now clear that these units derive directly from DHCA by peroxidase-mediated reactions.

Results and Discussion

Figure 1a shows a subplot of the sidechain region from a 2D HMQC-TOCSY NMR experiment on acetylated lignin isolated from the mutant pine, highlighting the new arylpropane-1,3-diacetates (bold) along with the previously identified DHCA units. Data from an APD model compound matches well.

Perhaps more diagnostic is the data from a 3D TOCSY-HSQC experiment. 2D F2-F3 planes from this experiment show a complete HSQC spectrum of any units bearing protons resonating at the frequency of the taken slice. In spectra as complex as those from lignins, finding unique resonances in the proton spectrum is often difficult. However, slices through each of the three sidechain protons in APD units show rather clear HSQC spectra of that unit, along with correlations from other units with protons resonating in the same regions. Thus, resonances at 2.2 ppm arise only from the new unit (β-proton) and DHCA (β-proton) (as well as the strong acetone solvent signal). Figure 1e is therefore a composite HSQC of those units, namely the acetone, as well as DHCA and APD. The plane through Ha (5.8 ppm, Fig. 1d) is quite clean but the TOCSY transfer between Hα and Hβ is poor (under the chosen acquisition conditions), giving rise to only weak Hβ/Cβ and Hγ/Cγ cross-peaks. γ-Protons in lignin seriously overlap; consequently the plane through an Hg (4.1 ppm, Fig. 1f) shows the nice correlations for the APD unit, but also with DHCA, lignin's β-aryl ether units, and the intense methoxyl. The observation of complete HSQC spectra for the ADP unit in all three planes corresponding to the APD sidechain protons (Figs. 1d-f), along with the evidence from the 2D-HMQC-TOCSY (Fig. 1a), provides sufficiently compelling proof of the structure in the isolated lignin from the CAD-deficient pine mutant.

As with other novel units found in lignins from transgenic or mutant plants, traces of the same components can be found in lignins from control plants. Figure 1b shows the α-C/H region of HSQC spectra of the mutant's lignin, where the β-aryl ether units and the strong new ADP unit appear most cleanly. Figure 1c shows the same region in lignin from a normal pine control. Although the peak is weak, it is diagnostic and, with the other correlations evident (not shown), well authenticated. The peak is also in lignins isolated from mature pine clear sapwood.

Where do APD units come from? They derive from DHCA **2** via the action of peroxidase and hydrogen peroxide, Fig. 2. The mechanism, via a vinylogous quinone methide, involves two H-radical abstractions. Abstraction from a benzylic CH_2 to produce quinone methides from phenoxy radicals has been noted previously. When DHCA is subjected to peroxidase-

H_2O_2, monomeric APD **1** as well as the range of homo- and crossed-dimers involving DHCA and APD are found.

Also evident in the HMQC-TOCSY spectrum, Fig. 1a, are (acetylated) ketones **3**. Products of benzylic alcohol oxidation are seen in various isolated lignins, notably from syringyl (3,5-dimethoxy-4-hydroxy-phenyl) units; they may arise during lignin isolation (particularly in the ball-milling step). Ketones **3** provide additional confirmatory evidence for the APD structures **1** described above.

Conclusion

A previously unidentified unit present in small quantities in normal lignins is a major component of the hydroxyphenylpropanoid polymeric component of a pine mutant deficient in CAD. Those units, arylpropane-1,3-diols, arise from dihydroconiferyl alcohol monomers by radical reactions, providing further evidence for the involvement of DHCA monomers in this plant's lignification. Lignin-biosynthetic-pathway mutants and transgenics provide a rich source of insight into details of the chemistry and biochemistry of hydroxyphenylpropanoid polymer formation. The full paper is available on our website at... http://www.dfrc.ars.usda.gov under Full-text Publications.

Figure 1. See next page.

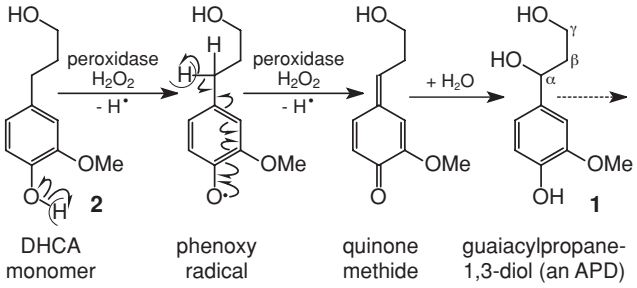

Figure 2. Scheme for production of APDs **1** from DHCA monomers **2** under lignification conditions

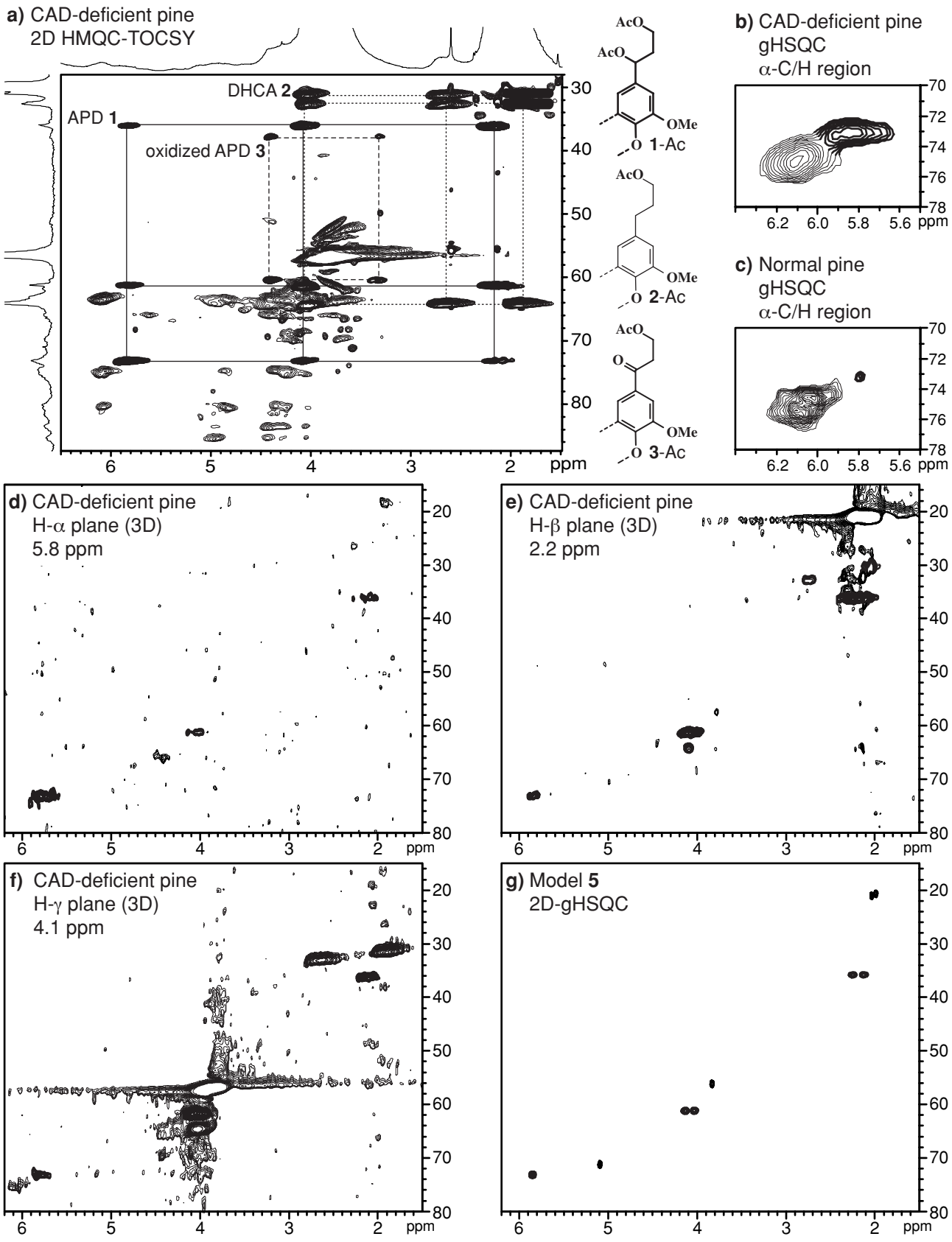

Figure 1. NMR spectra showing new APD structures **1** (bold) and derived benzylic ketone analogs **3**, along with DHCA units **2**. a-c, g are from 2D experiments; d-f are 2D planes from a gradient-selected 3D-TOCSY-HSQC experiment.

Detection and Determination of *p*-Coumaroylated Units in Lignins

F. Lu and J. Ralph

Introduction

In grasses hydroxycinnamic acids are highly involved in the lignification process during plant cell wall development. Ferulates in ferulate-polysaccharide esters are intimately incorporated into lignin via the free-radical coupling processes that typify lignification, producing strong lignin-polysaccharide cross-linking. Lignins in grasses also contain *p*-coumaric acid connected to lignin residues by ester linkages. Elucidation of the sites of *p*-coumarate attachment to lignin is important to understand mechanisms by which *p*-coumaric acid is incorporated into lignins and to understand its role in the plant.

NMR studies on isolated lignins from maize, and many other grasses reveal that *p*-coumarates are exclusively at the g-positions of lignin side-chains. Other studies suggested that *p*-coumarates are attached dominantly to syringyl units in grass lignins. Thioacidolysis detected *p*-coumarates on a maize lignin containing about 18% *p*-coumarate (Grabber *et al*., 1996), but cleaved some esters lowering its sensitivity to lignins with low contents of such esters.

DFRC (Derivatization Followed by Reductive Cleavage) is a procedure that produces analyzable monomers and dimers by cleaving α- and β-ethers in lignins. One advantage of the method is that g-ester groups on lignins remain largely intact. A modified DFRC protocol allowed successful proof of the occurrence of acetate groups on lignin side-chains in some species. Thus the method should allow us to confirm that *p*-coumarate groups are at the γ-positions of grass lignins, and determine their distribution on syringyl and guaiacyl β-ether units. Three isolated (bamboo, bromegrass and maize) lignins were subjected to DFRC degradation to provide information about sites of *p*-coumarate attachment and the likely pathway for its biosynthetic incorporation.

Experimental Procedures

Isolated Lignins. Lignins were isolated from bamboo, bromegrass, and maize. Plant material was ground in a Wiley mill (1 mm screen), and soluble phenolics, carbohydrates, and other components were removed by successive extractions with water, methanol, acetone, and chloroform. The ground wood was then ball-milled, treated with crude cellulases to degrade most of the polysaccharides, and extracted with 96:4 dioxane:water. Saccharides and metal ions were removed from the lyophilized crude lignin using 5 mM pH 8 EDTA.

The DFRC Procedure. The DFRC method is as described in Part 1 of a series of DFRC papers (Lu and Ralph, 1997b) and in the initial protocol (Lu and Ralph, 1997a) {all papers are on our website at http://www.dfrc.ars.usda.gov under the Full-Text Publications section}, but 4,4'-ethylidenebisphenol (Aldrich Chemical Co.) was used as the internal standard. Response factors for monomers **P**, **G** and **S** were 1.76, 1.37 and 1.46 respectively. {**P** is the DFRC monomeric product from *p*-coumaryl units, 4-acetoxycinnamyl acetate, not otherwise mentioned in this manuscript but included here for completeness}. Response factors for the acylated moieties, 4-acetoxy-3-methoxycinnamyl 4-acetoxyphenylpropionate **GP** and 4-acetoxy-3,5-dimethoxycinnamyl 4-acetoxyphenylpropionate **SP** were 2.58 and 3.00 respectively. Molar yields were calculated based on molecular weights of (underivatized) **P** =150, **G** =180, **S** =210; acetates **GP** = 412 and **SP** = 442 GC and GC-MS, and Synthesis. These details are described in the full paper (Lu and Ralph, 1999), available at http://www.dfrc.ars.usda.gov.

Results and Discussion

When the DFRC method was first developed, lignins representing softwoods, hardwoods, dicotyledons and grasses were used for testing its capabilities. Chromatograms of DFRC products from maize and bamboo lignins showed peaks in the dimeric compound region which were not present in products from other lignins. From NMR studies it was known that maize lignin contains large amounts of *p*-coumarate exclusively at the γ-positions of lignin side-chains. The new dimeric compounds were therefore suspected to be degradation products containing *p*-coumarate (or a derived product). Since DFRC does not affect lignin γ-esters in models, it seemed reasonable that this method could detect and determine *p*-coumarates attached to the γ-positions of β-aryl ether units in lignin (Fig. 1, lignin fragment **1**).

Compounds **GP** and **SP**, Fig. 1, were identified by GC-MS and comparison of their retention times with those of genuine compounds, which were synthesized and authenticated by NMR. Compound **SP** was a major component from ether extracts of DFRC degradation products of maize lignin, as confirmed by 2D NMR experiments. Mass spectra of **GP** and **SP** were diagnostic (Fig. 2). In each, the molecular ion was observed, and even-massed ion-radicals from one phenolic acetate loss [(M-42)+] were abundant. The most abundant ion fragment with m/z 107 is characteristic of the 4-hydroxybenzyl moiety from ring **P** (following loss of ketene from the acetate). Coniferyl and sinapyl alcohol fragment ions (m/z 180, 210) are prominent and other fragment ions are logical.

Syntheses of **GP** and **SP** allowed measurement of response factors so that *p*-coumarates in lignins released by DFRC could be quantified by GC. Table 1 shows DFRC results from lignins isolated from bamboo, maize, and bromegrass. In bromegrass, the amounts of esters were too low, but selected-ion mass spectrometry identified the sinapyl *p*-coumarate product **SP**; **GP** could not be detected. Evidently syringyl units **S** of bamboo and maize lignins are acylated by *p*-

coumarate to a much greater degree than guaiacyl units **G**. In bamboo and maize, the S/G ratios in the esters were 12- and 38-times greater than those of the normal monomers released by DFRC. More syringyl units than guaiacyl units are linked by labile β-aryl ethers, but this would only explain about a 2-fold difference in S/G molar ratios of DFRC products from both lignins. Studies with model compounds showed that acylation did not differentially affect the release of syringyl or guaiacyl units from β-aryl ethers. Preferential acylation of syringyl units by *p*-coumarate before their incorporation into lignin is therefore likely. Whole-cell-wall samples were not used in this study, but are expected to produce similar results, although some fractionation of the *p*-coumarate into the extractable lignins has been noted (Ralph *et al*., 1994). Whole-cell-wall studies will be aided by solid-phase extraction clean-up steps currently under development.

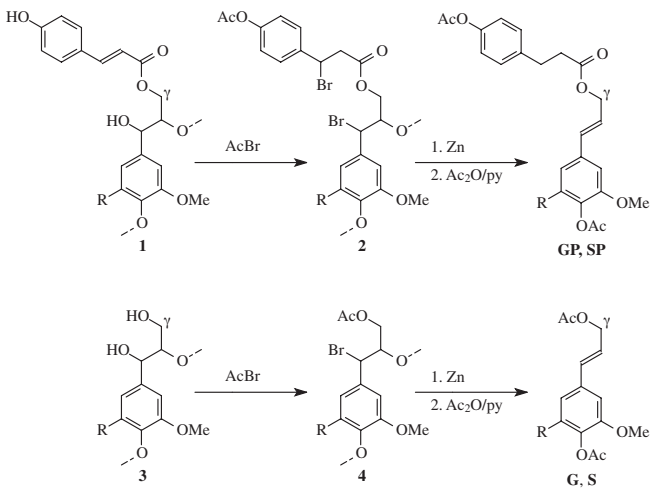

Figure 1. Pathway for formation of analyzable ester conjugates **GP**, **SP** from acylated β-aryl ether units **1** in lignins following DFRC.

Conclusions

Because DFRC leaves lignin γ-esters essentially intact, it is valuable for identifying esterified lignin components, and determining the sites of acylation. This study confirmed that grass lignins are acylated at the γ-position by *p*-coumarates; primarily syringyl units are acylated in maize, bamboo, and bromegrass.

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Table 1. Yields of monomers and acylated monomers from DFRC of bamboo, maize and bromegrass lignins.

	Monomers (mm/g)			*p*-coumarate products (mm/g)		
Sample	**G**	**S**	**S/G**	**GP**	**SP**	**SP/GP**
Bamboo lignin	504	222	0.44	6	32	5.33
Maize lignin	342	124	0.36	15	206	13.73
Bromegrass lignin	454	327	0.72	nd*	d	—

*nd = not detected, d = detected, by GC-MS.

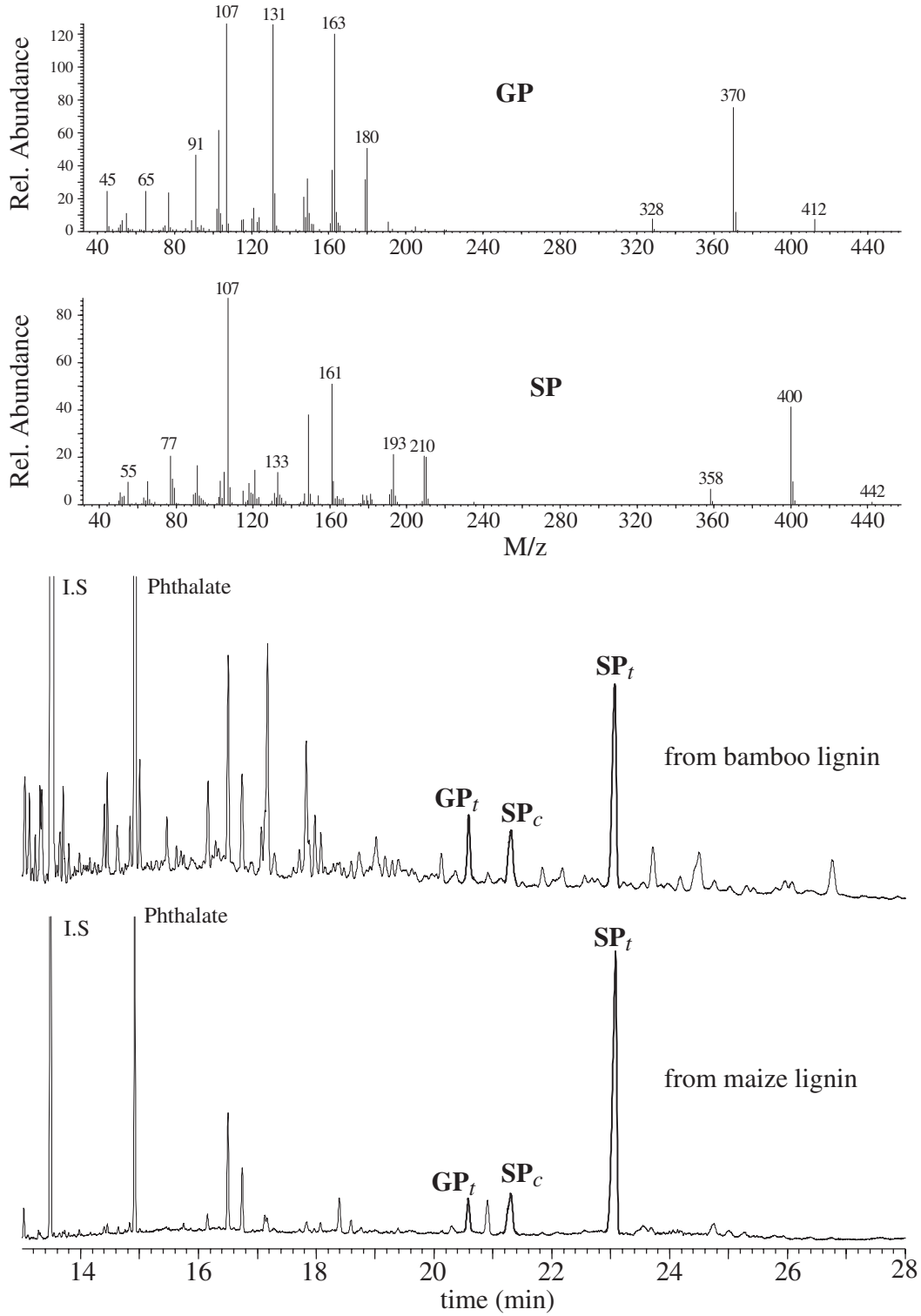

Figure 2. GC-FID chromatograms of DFRC dimers from bamboo and maize lignins. Products **GP** and **SP** from β-aryl ether γ-*p*-coumarate esters **1** are well resolved and easily detected. Their mass spectra are diagnostic. *c = cis, t = trans.*

The Effect of Down- and Up-regulation of Ferulate -5-hydroxylase on Lignin Content and Structure in Arabidopsis

J. Ralph, J.M. Marita, R.D. Hatfield and C. Chapple

Introduction

The biotechnological manipulation of lignin content and/or structure in plants is seen as a route to improving the utilization of plant cell wall polysaccharides in various agricultural and industrial processes. The aims range from enhancing cell wall digestibility in ruminants to reducing the energy demand and negative environmental impacts of chemical pulping and bleaching required in the papermaking process. To achieve these goals, many researchers have downregulated the expression of genes of the monolignol biosynthetic pathway in attempts to decrease lignin deposition. One of the more desirable strategies for modifying lignin is to produce a less cross-linked, syringyl-rich lignin. This modified lignin would have improved industrial and/or agricultural properties, but should still be fully functional in the plant. Although hardly a commercially useful forage plant, Arabidopsis is a useful model plant with a large part of the genome mapped.

Through the identification of the *fah1–2* mutant, Chapple's group at Purdue has shown that ferulate 5-hydroxylase (F5H) activity is required for syringyl lignin deposition in *Arabidopsis* (Chapple *et al*., 1992). The *f5h* gene has also been upregulated in the mutant to produce plants with very high syringyl contents. To date, the characterization of lignin in wild-type *Arabidopsis*, the *fah1–2* mutant, and the "35S–F5H" and "C4H–F5H" upregulated transgenics (Meyer *et al*., 1998) has employed only degradative methods. These techniques permit the analysis of only releasable units of the lignin polymer. In this study, we sought to more completely identify the nature and extent of the changes to lignin composition and structure in these plants by potent NMR methods.

Materials and Methods

General. NMR spectra were taken on a Bruker DRX-360 instrument fitted with a 5 mm ^{1}H/broadband gradient probe with inverse geometry (proton coils closest to the sample). The conditions used for all samples were ~ 40 mg isolated lignin in 0.4 mL acetone-d_6, with the central solvent peak as internal reference (δ_H 2.04, δ_C 29.80). The HMBC experiment was a standard Bruker implementation with proportion of guaiacyl residues, the overexpression of F5H is therefore strikingly effective at diverting the monolignol pool almost entirely into sinapyl alcohol.

1D ^{13}C-NMR Spectra. The syringyl/guaiacyl nature of lignins is also easily recognized in 1D ^{13}C (Fig. 2) and even ^{1}H-spectra (not shown). As noted above, syringyl units have symmetry. Their protonated aromatic 2/6-carbons are at ~105 ppm. This separates them from their guaiacyl counterparts gradient-selection and inverse- (^{1}H-) detection and a 100 ms long-range coupling delay. Carbon/proton designations are based on conventional lignin numbering.

Plant Materials and Lignin Isolation. Plant growth conditions, the *fah1–2* mutant, as well as the 35S–F5H and C4H–F5H *fah1–2* transgenic lines were previously described (Meyer et al., 1998). The ground stem material was ball-milled and extracted with 96:4 dioxane:water. The final yields of milled isolated lignin were 17% (by weight) of the lignin from the *fah1–2* mutant, 28% of the lignin from the C4H–F5H transgenic, 30% of the lignin from the 35S–F5H transgenic, and 25% of the lignin from the wild type. Following NMR of underivatized lignins (data not shown here), the isolated lignins were acetylated overnight with acetic anhydride/pyridine, and the solvents removed by co-evaporation with 95% ethanol. Traces of ethanol were removed by co-evaporation with acetone, followed by evaporation at reduced pressure (150 mTorr) for several hours. The acetylated lignins (~50 mg) were dissolved in acetone-d_6 (0.4 mL) for NMR.

Results and Discussion

Syringyl/Guaiacyl Nature from HMBC Spectra. The results of the previous chemical analysis (Meyer et al., 1998) of normal, mutant and transgenic lignins were readily verified by the NMR spectra. This is clearly revealed by sections of the long-range ^{13}C–^{1}H correlation (gradient-enhanced HMBC) experiments. Figure 1 shows the correlations between the a-protons of the major β-aryl ether units in lignins from the wild type, the *fah1–2* mutant, as well as the 35S–F5H and the C4H–F5H transgenics. As expected, the α-protons correlate with carbons β and γ of the sidechain and carbons 1, 2 and 6 of the aromatic rings; all of these carbons are within 3-bonds of the α-proton. What makes these correlation spectra useful is that the equivalent syringyl C–2/C–6 carbons, resonating at ~105 ppm, are well separated from their guaiacyl counterparts (for which C–2 and C–6 are different, at ~113 and ~120 ppm). Thus it is immediately clear that the wild-type control contains guaiacyl and syringyl (β–ether) units, with guaiacyl units predominating. The *fah1–2* mutant has almost no syringyl component; it is barely detectable at lower contour levels. These observations are consistent with data that indicate that F5H mRNA is below detectable limits in the *fah1–2* mutant (Meyer *et al*., 1996). The HMBC spectra show no significant differences between the wild type and 35S–F5H transgenic lignins. In contrast, the HMBC spectrum of lignin from the C4H–F5H transgenic line deviates strongly from that of the wild type. The lignin is extremely syringyl-rich. Only weak guaiacyl peaks could be discerned at lower levels. The guaiacyl level in the isolated

where protonated carbons 2, 5, and 6 range from ~110-125 ppm. The 1D spectra in Figure 2, with prominent peaks or regions labeled, indicate the differences between the lignins noted above. In particular the syringyl-rich C4H–F5H spectrum is particularly simple, being composed almost entirely of β-aryl ether units **A**, mostly with *erythro*-stereochemistry, and resinols **C**. As noted from 2D spectra, the *fah1–2* mutant spectrum has syringyl peaks that are at the limits of detection (denoted by arrow).

Discussion and conclusions from other types of NMR spectra can be found in the published paper (Marita et al., 1999), available on our website at http://www.dfrc.ars.usda.gov under full-text publications.

Conclusions

The NMR data presented here are consistent with previous biochemical data that indicated that F5H is a key regulatory point in the determination of lignin monomer composition (Meyer et al., 1998). The near absence of syringyl unit resonances in the lignin of the *fah1–2* mutant provides further evidence that F5H activity is required for syringyl lignin biosynthesis in *Arabidopsis*. Similarly, the domination of the NMR spectra by peaks attributable to sinapyl alcohol-derived subunits indicates that overexpression of the gene encoding F5H under the control of the C4H promoter leads to a lignin that is almost exclusively syringyl in nature.

Our results demonstrate that the manipulation of monolignol supply for the purposes of changing lignin subunit composition has been extremely successful in *Arabidopsis*. Lignin syringyl-guaiacyl compositions range from almost 100% guaiacyl in the F5H-deficient *fah1–2* mutant to nearly 100% syringyl (significantly higher than in any plant reported to date) in the best F5H-upregulated transgenic. Digestibility implications are still being assessed.

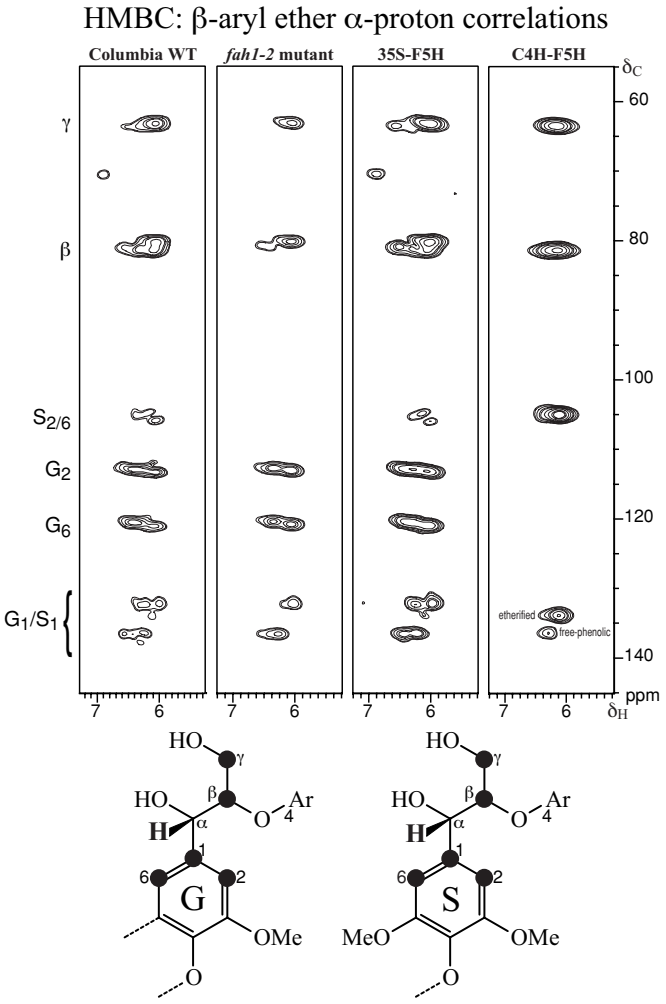

Figure 1. Gradient-selected 2D HMBC sub-spectra showing a-proton correlations in b-aryl ether units **A**. The syringyl/guaiacyl compositional changes are readily apparent in these spectra. The wild-type and 35S–F5H lignins are guaiacyl-rich syringyl/guaiacyl copolymers. In the *fah1–2* mutant, syringyl units are almost completely absent. When F5H is expressed under the control of the C4H-promoter, the lignin that is deposited is comprised almost exclusively of syringyl units. Structures at the bottom show the five carbons that are within 3-bonds of proton H–α, and therefore show HMBC correlations), and the lignin numbering conventions; G = guaiacyl, S = syringyl.

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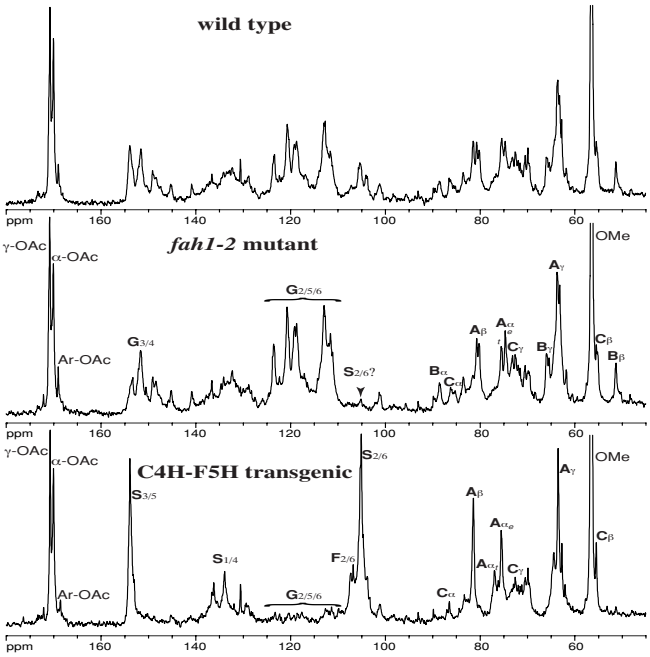

Figure 2. 1D ^{13}C NMR spectra of *Arabidopsis* lignins from a) the wild type, b) the *fah1–2* mutant, and c) the C4H–F5H transgenic. Major peaks are shown with their assignments; the letters **A**-**F** correspond to structures **A**-**F** as in a recent review; A = β-aryl ether, **B** = phenylcoumaran, **C** = resinol, **D** = dibenzodioxocin, **E** = α,β-diether (not present), **F** = α-keto-β-ether; **G** is used to represent a general guaiacyl unit, **S** a general syringyl unit; *e* = *erythro*-isomer, *t* = *threo*-isomer; oligosaccharides are present in these lignins.

Composition of Corn Pericarp/Seed Coat Tissues

R.D. Hatfield

Introduction

The outer surface of corn seeds, most frequently referred to as the seed coat, consists of several layers of cells that make up the pericarp and may be fused to the seed coat that immediately surrounds the endosperm and embryo. These tissues are important for the protection of the seed from insects, pathogens, and other environmental stresses but can also limit the digestibility and utilization of stored energy in rumen diets. Little is known about the chemical composition of corn pericarp/seed coat tissue and how this may impact their digestibility. This work was undertaken to determine the chemical composition of pericarp/seed coat tissue from field corn, popcorn and sweet corn.

Methods

Commercially available corn seeds were used for this study. Field corn was hybrid B73 x Mo17, sweet corn and popcorn were purchased at a local market (sweetcorn frozen Westpac Whole Kernel and popcorn Jolly Time White Kernel). For the field and pop corn samples 200 seeds were placed in 100 mL of 40 °C water and allowed to stand for 4 hours to soften the pericarp for easy removal. No softening was necessary for the sweetcorn. Pericarp/seed coat tissues were peeled from the endosperm using forceps and immediately frozen. All samples were ground to a fine powder in a freezer mill cooled with liquid nitrogen. Cell walls were isolated from these tissues using a hot amylase treatment to remove starch, followed by sonicated extraction with 80% ethanol, acetone, chloroform:methanol (1:1), and a repeat of the acetone wash. Samples were dried and analyzed for lignin, neutral sugars, total uronosyls, and hydroxycinnamic acids.

Results and Discussion

The seed coat is usually a one or two cell layer immediately surrounding the endosperm and embryo. Pericarp tissues on the other hand may be made up of several layers of cells with variable cell wall thickness. In this comparison popcorn has the thickest pericarp followed by B73 x Mo17 and with sweet corn having the thinnest pericarp. Cell wall composi tion among the three types of corn were generally similar (Table 1). Wall neutral sugars were similar among all the corn types. An interesting observation was the lower amounts of cellulose in these tissues (Table 1). Corn stem tissues typically contain 55 to 65% cellulose(glucose) and 30 to 40% xylan xylose) with only 3 to 5% arabinose. The xylans within these walls appear to be more highly substi-tuted with arabinose. It is also apparent that these tissues contain little or no lignin (1.5 to 3%). This analysis is based on recovery of an acid insoluble residue and may therefore contain structural proteins that would also be insoluble. Staining with phloroglucinol gave a negative response indicating no lignin.

A second major difference between these tissues and other corn tissues was the level of hydroxycinnamic acids (Table 2). The pericarp/seed coat tissues contained over double the level of ferulates typically found in corn stem tissues (1-1.5% of the cell wall). Typical corn walls will have a ferulate substitution ratio of 1:15-20(ferulate to arabinose) while these seed tissues have a ratio of 1:6. Levels of *p*-coumaric acid (*p*CA) were much lower as one might expect since most of the *p*CA is attached to lignin in grass cell walls. There were also higher amounts of ferulate dimers in these wall types. Although the total ferulates (monomers + dimers) was not different among the three corn pericarp/seed coats the proportion of dimers was different, with sweet corn having the lowest levels. This may be a due to the nature of the walls being thinner or possibly due to stage of development. The sweet corn was not at the same maturity level as the popcorn and the field corn. In any case the lack of lignin in these walls may be compensated for to some extent by increased levels of ferulates and ferulate cross coupling. An increased level of cross-linking within cell walls is generally going to result in decreased digestibility.

Table 1. Cell wall composition of pericarp/seed coat tissues isolated from different types of corn seeds.

Pericarp/ seed coat	mg/ gram cell wall					
	Lignin	Uronosyls	Arabinose	Galactose	Glucose	Xylose
B73 x Mo17	37.7 ± 5.8	40.7 ± 2.4	151.3 ± 0.9	58.1 ± 0.9	295.0 ± 12.1	306.7 ± 0.8
Pop corn	31.5 ± 6.0	50.7 ± 2.8	180.6 ± 1.9	50.6 ± 1.0	265.6 ± 6.2	327.4 ± 3.1
Sweet corn	15.2 ± 13.2	53.1 ± 0.5	188.0 ± 2.4	48.2 ± 0.2	282.4 ± 6.0	297.8 ± 2.6

Table 2. Hydroxycinnamate composition of pericarp/seed coat tissues isolated from different types of corn seeds.

Pericarp/ seed coat	mg/ gram cell wall			
	Total *p*CA	Total ferulates	Ferulate monomers	Ferulate Dimers
B73 x Mo17	1.8 ± 0.3	42.2 ± 0.8	28.8 ± 0.4	13.4 ± 0.4
Pop corn	2.6 ± 0.1	36.0 ± 5.2	27.2 ± 2.7	8.8 ± 2.5
Sweet corn	1.1 ± 0.1	41.9 ± 0.7	38.4 ± 0.6	3.5 ± 0.1

Value of Macerating Ryegrass Silage for Lactating Dairy Cows

G.A. Broderick, R.G. Koegel, T.J. Kraus and R.P. Walgenbach

Introduction

Improving the energy content of dietary forages would increase their value for dairy cows because less concentrate would need to be fed to maintain production. In previous research conducted at the Dairy Forage Center, we found that macerating alfalfa at time of mowing improved the Net Energy value of alfalfa silage by 5%. The main effect of maceration was to increase digestibility, particularly of the cell wall fractions – both neutral detergent fiber (NDF) and acid detergent fiber (ADF). Cool-season grasses such as ryegrass are used widely for feeding to dairy cows; these grasses are much higher in NDF and ADF than alfalfa. Increasing the rate of fiber digestion in grasses likely would increase both their Net Energy value and feed intake because fiber fill restricts dry matter (DM) intake. This report summarizes the findings from a trial that assessed the value of feeding Macerated ryegrass silage versus Control (unmacerated) ryegrass and alfalfa silages.

Materials and Methods

Ryegrass silage (RGS) was harvested using either a conventional mower-conditioner (Control) or the maceration machine (Macerated), field wilted to 35% DM and ensiled in AgBag silos during 1998. Degree of conditioning was assessed by comparing electrical conductivity of the leachates produced by processing Control and Macerated ryegrass (sampled prior to ensiling) in a blender. Control alfalfa also was harvested using the conventional mower-conditioner and stored in a bunker silo. A 3 x 3 Latin square lactation trial was conducted using 24 multiparous Holstein cows in early lactation. Cows were blocked by DIM and randomly assigned to diets containing (DM basis) 41% of either Control or Macerated ryegrass silage, or 51% alfalfa silage; the balance of diets were high moisture shelled corn, soybean meal and roasted soybeans (Table 1). Diets were fed once per day as total mixed rations for four-week periods before switching (total, 12 weeks). Digestibility of DM, organic matter (OM), NDF and ADF was estimated from fecal grab samples taken on the last day of each period using indigestible ADF as the internal marker. Daily milk production and DM intake for each cow were averaged over weeks 3 and 4 of each period. Milk content and yield of fat, protein, lactose, and SNF was determined from milk samples taken at PM and AM milkings mid-way through weeks 3 and 4. Milk urea N (MUN) also was determined in these samples. Body weight (BW) was measured once daily for three consecutive days at the start of the trial and at the end of each period. Cows were injected biweekly with rBST. Data were analyzed using the general linear models procedures of SAS.

Results and Discussion

The two RGS fed in this trial were harvested from alternate windrows from the same fields; however, Macerated RGS contained more NDF, ADF, indigestible ADF, and less crude protein (CP) then Control RGS (Table 1). These differences suggested that there was greater leaf loss during harvest of the Macerated RGS. In earlier studies, similar CP, NDF, and ADF contents were observed in Control and Macerated alfalfa silages. As expected, the alfalfa silage was lower in NDF and higher in CP, ADF, and indigestible ADF. The NPN content of both Control silages was about 60% of total N; Macerated RGS had about 10 percentage units less NPN. Previously, we found that maceration reduced silage NPN; lowering NPN tends to improve CP utilization. Diets were intended to be isonitrogenous but that containing Macerated RGS had 0.8-percentage unit less CP (Table 1). Also, the Control RGS diet was > 1 percentage unit lower in NDF than the macerated RGS.

Animal performance data from this trial are summarized in Table 2. Overall, there were no differences ($P \geq 0.34$) in DM intake, BW gain, milk yield, milk yield/DM intake, milk composition, and yield of milk components with feeding of Control or Macerated RGS. Previously, we observed that macerating ryegrass forage improved ruminal in situ digestibility in some cases but not others. There was a 10% increase in leachate electrical conductivity for Macerated versus Control ryegrass. While this represents a degree of conditioning greater than the conventional mower-conditioner, it is relatively low on an overall scale. In the past, macerating alfalfa has increased leachate conductivity by 60% versus Control. A striking finding in this trial was that DM intake was about 8 kg/d greater on alfalfa silage than on either RGS diet. As a result, BW gain, yield of milk and milk components, and milk protein, lactose, and SNF content all were greater ($P \leq 0.05$) on alfalfa silage. The only exception to this trend was the substantially better ($P < 0.01$) milk yield/DM intake on the two RGS diets. Concentrations of MUN were not influenced by silage source in this trial.

Effect of forage source on apparent nutrient digestibility is summarized in Table 3. Except for ADF, there were no differences ($P \geq 0.12$) in nutrient digestibility between feeding Control and Macerated RGS. The lower ($P = 0.02$) apparent digestibility of ADF on Macerated RGS – despite similar yields of milk and milk components at equal DM intake – probably reflected the slightly lower cell wall content of the Control RGS harvested in this trial (Table 1). Apparent digestibilities for all five nutrients measured were lower ($P < 0.01$) on alfalfa silage. The NEL requirement for maintenance, BW gain, and milk production was computed from NRC tables; these values – which were mainly a

function of milk yield – also are in Table 3. Expressing NEL requirements per unit of digestible DM consumed yielded values for the Control and Macerated RGS diets that were 5 and 9% greater than that for alfalfa silage. The similarity of this result was surprising in view of the 30 and 25% greater milk yields/DM intake observed on the diets containing, respectively, Control and Macerated RGS (Table 2). This finding suggested that the greater yields of milk and milk components on alfalfa silage were driven mainly by the greater intakes on that diet.

Summary and Conclusion

Macerating ryegrass immediately after cutting, before ensiling the forage, reduced NPN formation in the silo but did not otherwise improve forage utilization by lactating dairy cows. In this study, the maceration process tended to reduce CP and increase fiber, suggesting greater leaf loss during harvest of Macerated versus Control RGS. Intake of DM was dramatically greater on alfalfa silage than on RGS; yield of milk and milk components also was much higher on alfalfa. Greater milk yield/DM intake on RGS than alfalfa silage was explained by greater nutrient digestibilities on the two RGS. A computation of the Net Energy supplied per unit of digestible DM consumed indicated that diets containing RGS and alfalfa silage were similar. The very low feed intakes and poor performance on both RGS diets in this trial indicated that RGS was, relative to alfalfa silage, an inferior forage for high producing lactating cows.

Table 1. Composition of silages and diets fed during lactation trial.

Item	A Control RGS	B Macerated RGS (% of DM)	C Alfalfa Silage
Silage composition			
Organic matter	84.9	85.2	89.1
NDF	49.5	51.2	43.5
ADF	29.2	30.7	34.7
Indigestible ADF	14.2	17.7	28.2
Crude protein	18.4	17.1	21.7
NPN (% of total N)	59.6	49.7	60.9
Dietary ingredients			
Control ryegrass silage	40.6	. . .	. . .
Macerated ryegrass silage	. . .	40.6	. . .
Control alfalfa silage	. . .	. . .	51.2
Rolled high moisture shelled corn	44.8	44.8	39.5
Soybean meal	8.7	8.7	3.2
Roasted soybeans	5.4	5.4	5.3
Dicalcium phosphate	0.1	0.1	0.4
Salt	0.3	0.3	0.3
Vitamin-mineral concentrate[1]	0.1	0.1	0.1
Dietary composition			
Crude protein	18.2	17.4	18.2
Organic matter	90.8	90.2	92.4
NDF	27.7	29.2	28.8
ADF	14.8	15.6	20.6
Indigestible ADF	6.5	7.6	15.0
P[2]	0.40	0.40	0.40

[1]Provided (per kg of DM): Zn, 56 mg; Mn, 46 mg; Fe, 22 mg; Cu, 12 mg; I, 0.9 mg; Co, 0.4 mg; Se, 0.3 mg; vitamin A, 6440 IU; vitamin D, 2000 IU; and vitamin E, 16 IU.
[2]Computed from NRC tables.

Table 2. Performance of lactating cows fed diets containing forage harvested as either control ryegrass silage (CRGS), macerated ryegrass silage (MRGS), or control alfalfa silage (AS).

	Dietary forage					Contrasts[2]	
Variable	CRGS	MRGS	AS	SE[1]	$P > F^2$	CRGS vs. MRGS	AS vs. RGS
DMI, kg/d	16.8	17.0	25.2	0.5	< 0.01	0.85	< 0.01
Milk, kg/d	35.6	34.5	41.1	0.5	0.06	0.74	0.04
Milk yield/DMI	2.15	2.06	1.65	0.04	0.01	0.53	< 0.01
BW gain, kg/d	0.28	0.50	0.81	0.28	0.10	0.34	0.05
3.5% FCM, kg/d	31.3	31.2	38.0	0.8	0.04	0.97	0.01
Fat, %	2.80	2.94	3.08	0.09	0.39	0.52	0.23
Fat, kg/d	0.98	1.00	1.25	0.04	0.03	0.86	< 0.01
Protein, %	3.16	3.18	3.31	0.05	0.06	0.71	0.02
Protein, kg/d	1.11	1.09	1.35	0.03	0.01	0.77	< 0.01
Lactose, %	4.76	4.75	4.93	0.03	0.03	0.91	< 0.01
Lactose, kg/d	1.68	1.64	2.01	0.03	0.04	0.74	0.01
SNF, %	8.72	8.73	9.06	0.07	0.02	0.90	< 0.01
SNF, kg/d	3.08	3.00	3.69	0.05	0.03	0.74	< 0.01
MUN, mg/dl	11.1	10.2	10.8	0.43	0.42	0.21	0.77

[1]

SE = Standard error.

[2]Probability of a significant effects of diet and orthogonal contrasts (error = cow(square)).

Table 3. Effect of feeding control ryegrass silage (CRGS), macerated ryegrass silage (MRGS), or control alfalfa silage (AS) on apparent digestibility and utilization of nutrients.

	Dietary forage					Contrasts[2]	
Nutrient	CRGS	MRGS	AS	SE[1]	$P > F^2$	CRGS vs. MRGS	AS vs. RGS
	----------(%)----------						
Dry matter	77.7	76.1	66.0	1.2	< 0.01	0.28	< 0.01
Organic matter	79.4	78.5	68.1	1.1	< 0.01	0.51	< 0.01
NDF	66.1	63.2	42.2	1.8	< 0.01	0.12	< 0.01
ADF	64.7	60.1	40.8	1.9	< 0.01	0.02	< 0.01
Crude protein	71.7	69.5	61.0	1.3	< 0.01	0.28	< 0.01
Net energy computations							
Required,[3] Mcal/d	33.6	34.6	41.5	. . .	. . .	. . .	. . .
DDM Intake,[4] kg/d	13.0	13.0	17.0	. . .	. . .	. . .	. . .
NEL/DDM, Mcal/kg	2.58	2.67	2.45	. . .	. . .	. . .	. . .
Relative NEL/DDM[5]	1.05	1.09	1.00	. . .	. . .	. . .	. . .

[1]SE = Standard error.

[2]Probability of a significant effects of diet and orthogonal contrasts (error = cow(square)).

[3]Requirements for NEL for maintenance (582 kg BW), BW gain, and Milk yield computed using NRC tables.

[4]Intake of digestible DM computed from DM intake (Table 2) and apparent DM digestibility.

A Corn Mutant with Reduced Ferulate Esters for Lignin/Polysaccharide Cross-Linking has Improved Cell-Wall Degradability

H.G. Jung, W. Ni, and R.L. Phillips

Introduction

The USDFRC Cell Wall Group has demonstrated the importance of ferulic acid mediated cross-linking of lignin and arabinoxylans in the cell walls of grasses to wall degradability using an *in vitro* corn cell-culture model system. Last year we reported that selection for reduced cross-linking in smooth bromegrass improved cell-wall degradability. In an attempt to use a molecular biology approach to achieve the same result in corn, we reported in the USDFRC 1996 Research Summaries that a mutant corn line had been created through transposon mutagenesis. This mutant corn line (3503) has reduced concentrations in seedling leaves of the ferulate ester precursors to cross-link formation. This mutation is heritable and appears to function as a recessive allele. Because of the presence of other unrelated transposon-induced mutations, we were previously unable to accurately access the impact of this mutation on ferulate cross-linking and cell-wall degradability in mature plants. Our expectation is that reducing the amount of ferulate esters in immature tissue will cause a decrease in cross-linking and improve cell-wall degradability of mature tissues.

Materials and Methods

The original 3503 mutant line was backcrossed to one of its parents (W23) for two generations, after which the progeny were selfed. Ten selfed lines were planted in the field in 1998 for a ferulate ester phenotype segregation analysis. The first true leaf was collected from corn seedlings when the third true leaf had emerged. Ferulate esters were extracted with alkali and quantified by HPLC analysis. At physiological maturity, three lines which segregated for ferulate ester concentrations at the seedling stage were harvested. The five lowest and five highest ferulate ester concentration plants in these lines were selected for a detailed cell-wall composition and degradability analysis. Corn stover was separated into leaf blade, leaf sheath, and stem for analysis. Only the leaf blade data will be reported here. Cell-wall components measured included: polysaccharide sugar residues, Klason lignin, and ester- and ether-linked ferulic acid. Leaf blades were fermented with rumen fluid for 24- and 96-h, and the residues were analyzed for residual cell-wall polysaccharide components.

Results and Discussion

Four of the 10 lines were judged to be segregating for the low ferulate mutant phenotype. This result is close to the 50% segregation incidence expected for a recessive allele after two generations of backcrossing. While a 3:1 segregation ratio (normal vs. low ferulate concentration) among plants within a line would be predicted for a recessive trait, poor germination and the presence of continued transposon activity (as shown by the presence of chlorophyll deficient seedlings) precluded such segregation ratio analysis. Figure 1a illustrates the difference in concentration between individual plants of three segregating lines classified as having high or low ferulate ester levels in seedling leaves. At physiological maturity, only two of the three lines still exhibited a difference between plants in these two seedling classifications and this difference was reduced in magnitude (Fig. 1b). Only minor differences in mature leaf blade cell-wall concentration and composition were found between sibling plants within a line. Surprisingly, the mutant plants in line 101-13-2 actually had more ferulate cross-links (measured as ethers) than the normal plants. However, for the two lines that maintained the seedling difference in ferulate esters at maturity we observed increased cell-wall polysaccharide degradability after 96-h fermentations. No differences were observed after 24-h fermentations (data not shown).

Conclusions

From this limited analysis of plants exhibiting the mutant low ferulate ester phenotype in seedling leaves compared to their normal phenotype siblings, it appears that reduction in the number of potential ferulate cross-linking sites does improve cell-wall degradability of mature plants as predicted. If the mutated gene can be cloned, then it should be possible to transfer the reductions in ferulates and improved degradability to other corn lines and grasses through biotechnology. The apparent contradiction in our data (more ferulate ether cross-links in some mutant line plants) may be caused by the role of ferulate esters as nucleation sites in lignin deposition and, because lignin concentration was not reduced by this mutation, a higher proportion of the remaining ferulate esters became involved in cross-link formation during lignin deposition. We hypothesize that reduced ferulate esters in immature tissues will lead to formation of fewer diferulate cross-links between arabinoxylans and subsequent cross-linkage to lignin. Therefore, the improvement in cell-wall degradability due to the ferulate ester mutation may be due to reduced diferulate cross-linkage of lignin to arabinoxylan. This possibility is under investigation.

Table 1. Cell-wall composition and 96-h degradability of mature leaves from three corn lines segregating for the low ferulate ester mutation in seedling leaves.

	Line 101-1-4		Line 101-13-2		Line 101-14-7	
Trait	Normal	Mutant	Normal	Mutant	Normal	Mutant
Composition						
	- - - - - - - - - - - - - - - - - - g kg $^{-1}$ OM - - - - - - - - - - - - - - - -
Cell wall	731	718	616	686	650	701
	- - - - - - - - - - - - - - - - g kg $^{-1}$ Cell Wall - - - - - - - - - - - - - - - - - -
Glucose	420	408	402*	434	397	412
Xylose	255	255	258	259	270	268
Arabinose	57*	63	68*	58	69*	62
Galactose	34	38	43*	32	37	33
Uronics	43	45	51*	46	46	46
Lignin	155	151	142	136	137	138
Ferulate						
esters	5.13	5.24	5.51*	4.40	6.28*	5.12
ethers	6.41	6.43	5.90*	6.82	6.22	6.32
	- - - - - - - - - - - - - - - - - - % - - - - - - - - - - - - - - - - -
Degradability						
Polysaccharide	68.7	66.4	63.4*	71.7	66.7*	70.0

*Normal and mutant phenotypes, within a line, differ (P<0.10).

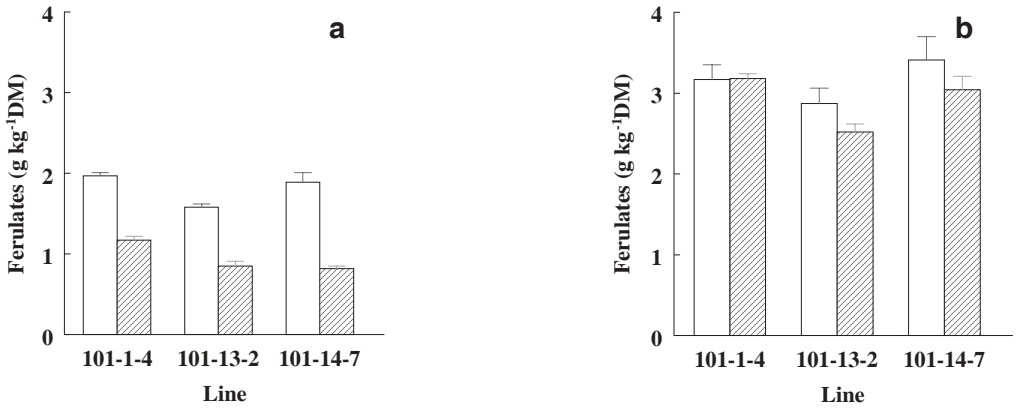

Figure 1. Ferulate ester concentration of seedling (a) and mature (b) leaf blades of low (cross-hatched) and high (open) plants from three lines segregating for the 3503 mutant phenotype.

Cross-Linking of Grass Cell Walls by Ferulate Dimerization and Incorporation Into Lignin

J.H. Grabber, J. Ralph, R.D. Hatfield and G. Wende

Introduction

Ferulic acid is ester linked to α-L-arabinosyl sidechains on grass xylans. Xylans become cross-linked by peroxidase-mediated coupling of ferulate into dehydrodimers. Biomimetic lignification systems and ferulate-lignin isolates from grasses have revealed that ferulate and diferulate esters copolymerize with monolignols, thereby cross-linking xylans to lignin. These cross-links probably contribute to wall stiffening and growth cessation in grasses and to poor degradation of grass walls by hydrolytic enzymes. The fate of ferulate and diferulates in walls are difficult to track because ferulate deposition, dimerization and copolymerization into lignin are overlapping processes during cell wall formation in grasses. This difficulty is further compounded by our inability to fully recover or characterize, by solvolytic or spectroscopic methods, ferulates in lignified walls. In this study, feruloylated primary walls from nonlignified maize cell suspensions and ethyl esters of ferulates and diferulates were used as model systems to isolate and study the dimerization of ferulates and their subsequent incorporation into lignins.

Methods

A dilute H_2O_2 solution was added to walls isolated from maize cell suspension to stimulate oxidative coupling of ferulate into diferulates by wall bound peroxidases. Walls were then slowly lignified *in situ* by adding solutions of coniferyl alcohol and H_2O_2. In a separate experiment, coniferyl alcohol and ethyl esters of (*E*)-ferulate, (*E*)-8–5-coupled diferulate, or (*E*)-5–5- coupled diferulate were polymerized *in vitro* by H_2O_2 and maize peroxidases. Samples were subjected to alkaline hydrolysis at room temperature or at 170 °C. Alkali-labile acids were analyzed by GC-FID and GC-MS.

Results and Discussion

In vitro and *in situ* experiments indicated that xylans were extensively cross-linked at the onset of lignification by dimerization of up to 60% of cell-wall ferulates. *In vitro* and *in situ* dimerization of ferulates yielded mostly 8–5- and other 8-coupled products. Significant 5–5-coupling was observed only within cell walls. Most ferulate and diferulate isomers began incorporation at the start of lignification and the final extent of incorporation usually exceeded 90% (Figure 1 and Table 1.). Incorporation data were best described by a dual-pool exponential model. Ferulate and 5–5-coupled diferulate reached 90% incorporation after coniferyl alcohol additions of only 86 mg g^{-1} and 56 mg g^{-1}, respectively. In contrast, coniferyl alcohol additions of >200 mg g^{-1} were required for 90% incorporation of 8–8-, 8–*O*–4, and 8–5-coupled diferulates. Incorporation of (*E*)-isomers was also more rapid than (*Z*)-isomers. Only 34% of the ferulates and 26 to 66% of the diferulates incorporated into lignins were released by alkaline hydrolysis of ether linkages (Table 2). This confirms that a significant proportion of the cross-linked structures involve alkali-resistant styryl ether and C-C linkages.

Conclusions

During lignification, primary walls in maize are extensively cross-linked by oxidative coupling of ferulate monomers into dehydrodimers and by incorporation of ferulate monomers and dehydrodimers into lignin. Ferulate monomers and dehydrodimers differ substantially in their propensity to copolymerize with coniferyl alcohol and to form hydrolysable ether-linked structures with lignin. As a result, solvolytic cleavage of ether-linkages probably provides a poor estimate of ferulate and diferulate cross-linking in

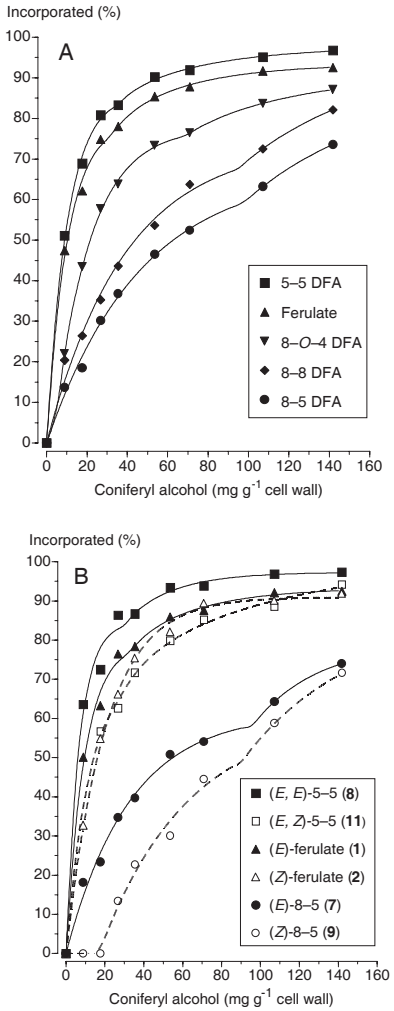

Figure. 1. Incorporation of ferulate and diferulate (DFA) structural isomers (A) and geometric isomers (B) during lignification of primary maize walls with coniferyl alcohol. Prior to lignification, ferulates were pre-dimerized by

Table 1. Kinetic parameters describing the relationship between the incorporation of ferulate (FA) and diferulates (DFA) into lignin and the quantity of coniferyl alcohol polymerized into walls.[1]

Constituent	L1	*k*1	A1	L2	*k*2	A2	Fit
Overall incorporation							
FA and DFA	0.0	0.058	71.3	57.2	0.011	23.7	0.995
Incorporation of structural isomers							
FA	0.0	0.097	79.3	31.4	0.026	14.1	0.998
5–5 DFA	0.0	0.097	86.2	35.1	0.022	11.5	0.999
8–*O*–4 DFA	2.9	0.055	77.7	68.5	0.015	14.0	0.999
8–8 DFA	0.0	0.026	74.0	94.3	0.011	24.0	0.994
8–5 DFA	0.0	0.022	67.4	97.4	0.012	22.7	0.997
Incorporation of geometric isomers							
(*E*)-FA	0.0	0.105	79.3	32.3	0.028	14.0	0.997
(*Z*)-FA	0.7	0.058	86.2	55.5	0.016	7.8	0.999
(*E,E*)-5–5 DFA	0.0	0.144	84.6	29.3	0.040	12.7	0.993
(*E,Z*)-5–5 DFA	0.0	0.083	72.3	26.8	0.013	27.1	0.999
(*E*)-8–5 DFA	0.0	0.031	61.4	95.9	0.031	17.7	0.996
(*Z*)-8–5 DFA	16.8	0.022	61.2	90.5	0.016	25.9	0.995

[1] Data were fitted to the dual pool exponential model $y = A1[1 - e^{-k1 \times (CA - L1 - |L1 - CA|)}] + A2[1 - e^{-k2 \times (CA - L2 - |L2 - CA|)}]$ where, CA = coniferyl alcohol added to cell walls (mg g^{-1}), A1 = rapidly incorporated pool (%), *k*1 = rate of A1 incorporation, L1 = CA added prior to A1 incorporation, A2 = slowly incorporated pool (%), *k*2 = rate of A2 incorporation, and L2 = CA added prior to A2 incorporation. Fit was calculated as 1 – (residual sum of squares/total degree-of-freedom corrected sum of squares).

Table 2. Fate of ferulate and diferulates (DFA) during polymerization of coniferyl alcohol into nonlignfied maize walls[1]. Cell walls from two experiments were lignified to an average Klason lignin content of 138 mg g^{-1}.

		Ferulate	8-8 DFA	8-5 DFA	8-*O*-4 DFA	5-5 DFA
Nonlignified walls	**(mg g^{-1})**	**7.5 ± 0.2**	**1.9 ± 0.2**	**6.3 ± 0.8**	**1.9 ± 0.2**	**2.1 ± 0.3**
Incorporated into lignin	(mg g^{-1})	6.9 ± 0.2	1.5 ± 0.2	4.5 ± 0.8	1.6 ± 0.2	2.0 ± 0.3
	(%)	91.9 [a]	78.8 [bc]	71.5 [c]	85.2 [ab]	93.5 [a]
Ether linked to lignin	(mg g^{-1})	2.4 ± 0.3	0.8 ± 0.1	—	1.0 ± 0.1	0.5 ± 0.2
	(%)	34.0 [b]	56.2 [a]	—	66.0 [a]	26.4 [b]

[1] Due to modifications of some geometric and structural isomers during hydrolysis, only sums of isomers are presented. The release of ether-linked 8–5-coupled diferulates could not be estimated.
[2] Percentages not followed by the same letter are significantly different according to LSD ($p = 0.05$).

Ruminal Fermentation of Cellulose/Xylan Composite Structures

P.J. Weimer, J.M. Hackney, R.D. Hatfield and H.J. -G. Jung

Introduction

Recent studies have emphasized that matrix interactions between polysaccharides and lignin, particularly those mediated by covalent cross-linkages involving phenolic acids, are a primary mechanism imparting recalcitrance of plant cell walls to biodegradation. Reducing this crosslinking by chemical or genetic methods represents a promising approach to increasing forage cell wall digestibility. It is not clear, however, which other cell wall structural features provide additional limitations to biodegradability. Because cellulose is found in intimate association with various hemicelluloses in plant cell walls, and because some plant cell walls are degraded without preferential loss or accumulation of specific component monosaccharides, the interactions among the wall polysaccharides may have an effect of the degradation of individual component polysaccharides.

The purpose of this study was to determine if matrix interactions among the polysaccharides, independent of lignin, provide a secondary mechanism of recalcitrance that could restrict the rate of plant cell wall degradation below the rates of degradation of the pure polysaccharides. Direct studies of the effects of polysaccharide interactions on cell wall degradability are complicated by the great variety of polysaccharides present in plant cell walls, and by the presence of lignin and phenolic acids in even very young plant tissue. Consequently, we took an alternative experimental approach in which we used the cellulose-synthesizing bacterium *Acetobacter xylinum* to prepare cellulose/xylan composites, and we then compared the kinetics of digestion of these composites by mixed ruminal microorganisms to those of the component polysaccharides and a nonassociated mixture of these polysaccharides.

Materials and Methods

Xylan was sequentially extracted from ground, flue-cured tobacco stalks using 5% and 24% (w/v) of KOH. The extracted xylan was precipitated in 80% ethanol and recovered by centrifugation; this fraction was designated TSX2. Bacterial cellulose was synthesized by cultures of *A. xylinum* ATCC 53524 grown in Hestrin-Schramm medium in a shaking water bath (30 °C, 250 rev/min) for 3 or 5 d. Cellulose was purified by a stepwise procedure involving vacuum filtration; Soxhlet extraction with methanol, then with chloroform/methanol; washing with methanol, then with water; and boiling in 1% NaOH, then in water. The final product was thoroughly rinsed with water and then lyophilized. The bacterial cellulose/xylan composite was obtained in a similar fashion as the bacterial cellulose, except the Hestrin-Schramm growth medium was supplemented with 0.2% (w/v) of TSX2. The composite will hereafter be referred to as BCX.

In vitro fermentations of the polysaccharides were conducted in serum vials placed in a chamber (39 °C) containing hypodermic needle-fitted pressure transducers that permitted determination of the kinetics of gas production from individual fermentation vials. Vials contained 10-22 mg of substrate, 5.7 mL of McDougall buffer and 0.3 mL of cysteine-sulfide reducing agent, under a CO_2 gas phase. Vials were inoculated with diluted ruminal fluid, to provide a ruminal fluid concentration equivalent to 20% (v/v). Following corrections for changes in ambient barometric pressure and for gas production in blank vials lacking added substrate, the data were fit to several models using the PROC NLIN routine of the SAS statistical analysis software package. At the end of the 48 h incubation period, nongaseous fermentation products were quantified by HPLC.

Results and Discussion

Tobacco stalk xylan (TSX2) was pure white in color, and contained 7% (molar basis) of non-xylosyl residues. Linkage analysis indicated that the xylose residues were 1,4-linked, with relatively little branching. Based on the content of terminal Xyl residues, the xylan had approximate degree of polymerization of 100.

Neutral sugar analysis revealed that the cellulose/xylan composites purified from these cultures contained molar xylose/glucose ratios as high as 0.24 (~ 18% xylose on a molar basis). Treatment of the composites with 2 N trifluoroacetic acid for 90 min at 120 °C did not result in significant release of neutral sugars. X-ray diffraction patterns of the cellulose/xylan composite were virtually identical to those of the bacterial cellulose, and revealed that both the pure cellulose and the composite were highly crystalline. These data indicate that cellulose and xylan in the composite were intimately associated, thus resembling the relationship of these polysaccharides in the native plant cell wall.

Progress curves for gas production with time from the various substrates are shown in Fig. 1. Gas production from the soluble TSX2 substrate proceeded almost immediately upon inoculation, and was complete within 4 h, while gas production from the celluloses and composites proceeded

more slowly, and only after a substantial lag period. Application of the data to a dual pool exponential model with two lag times provided the best fit of the data and allowed a quantitative comparison of the fermentation characteristics of the substrates (Table 1). TSX2 displayed two fractions that digested at considerably different rates, and the more abundant pool was digested with first-order rate constant of 1.16 h^{-1}. The rate constant for gas production from bacterial cellulose (BC) was twice as great as that of Sigmacell 50 (SC50), a microcrystalline cellulose derived from cotton, and its lag time was significantly shorter. These differences probably reflect differences in the relative proportions of the Iα.Iβ allomorphs of these crystalline substrates. The total amount of gas produced was slightly greater from the BC than from SC50; this difference may be due in part to the different endproduct ratios that resulted from the two fermentations. Fermentation of BC resulted in a net ratio of acetate to propionate (A/P) that was 2.3-fold greater than that resulting from fermentation of SC50 (4.2 vs. 1.5, $P < .05$).

Gas production from an unassociated 10:1 (w/w) mixture of bacterial cellulose and TSX2 (BC+TSX2) was similar to that of the BC alone, except for a slightly elevated gas production earlier in the fermentation, due to the relatively rapid fermentation of the small amount of TSX added to the mixture. The extent of gas production from BC+TSX2 was similar to the sum of these two components. The BCX composite and the unassociated mixture of the two components (BC+TSX2) displayed similar rate constants and lag times and similar A/P ratios, although total gas production was slightly lower for the BCX composite (Fig. 1).

The BCX composite was degraded at a rate similar to that of a mixture of its two non-associated components. This suggests that the ruminal population is well adapted toward degrading cellulose-containing structures in intimate association with xylan. These results are consistent with observations that the lag time for degradation of cellulose of ground forages is much shorter than for pure celluloses. Thus, while matrix interactions (e.g., hydrogen bonding) between cellulose and xylan may strengthen the plant cell wall, these interactions apparently do not inhibit the degradability of cellulose.

Conclusions

Removal of the primary constraints in plant cell wall degradation (crosslinking of polysaccharides to phenolics and lignin) enhance digestibility, without polysaccharide matrix interactions between cellulose and xylan (¾ the two most abundant cell wall polysaccharides ¾) providing secondary limitations to digestibility. Examination of interactions among less abundant cell wall polysaccharides may reveal whether the lack of inhibition observed in the digestion of cellulose/xylan composite applies to other polysaccharide interactions as well.

Table 1. Kinetic parameters for gas production from polysaccharides, fit to a two-pool exponential model having two discrete lag times.[a]

	A	B	k_1	k_2	L_1	L_2
Substrate[b]	(mL gas g^{-1})	(mL gas g^{-1})	(h^{-1})	(h^{-1})	(h)	(h)
SC50	21.4^{d}	341.6^{c}	0.110^{d}	0.074^{c}	0.54^{c}	7.99^{c}
BC	80.3^{cd}	274.3^{cd}	0.140^{d}	0.152^{d}	0.34^{c}	6.20^{de}
TSX2	105.5^{c}	67.5^{e}	1.038^{c}	0.190^{d}	0.03^{c}	$< 0.01^{f}$
BC+TSX2	90.2^{cd}	286.0^{cd}	0.125^{d}	0.155^{d}	0.66^{c}	6.62^{d}
BCX	108.3^{c}	243.7^{d}	0.120^{d}	0.179^{d}	0.39^{c}	5.32^{e}

[a]A and B, gas produced from rapidly and slowly digesting pools, respectively; k_1 and k_2, rate constant for rapidly and slowly digesting pools, respectively; L_1 and L_2, lag times for rapidly and slowly digesting pools, respectively.
[b]SC50, Sigmacell 50 microcrystalline cellulose; BC, bacterial cellulose; TSX2, tobacco stalk; BC+TSX2, 10:1 mixture of BC and TSX2; BCX, cellulose/xylan composite.
[cdef] Means with different superscripts differ ($p<.05$)

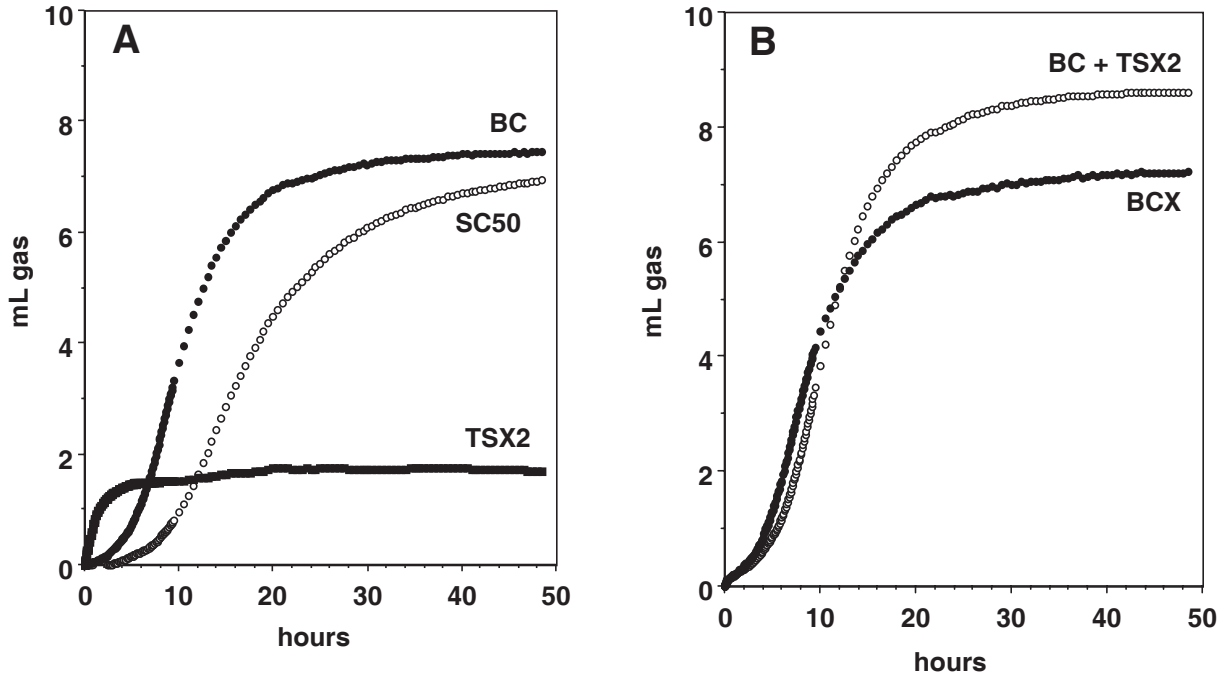

Figure 1. Time course of in vitro gas production from polysaccharides. See Table 1 for identification of substrates.

Selection of a Highly Monensin-Resistant *Prevotella bryantii* Sub-population with Altered Outer Membrane Characteristics

T.R. Callaway and J.B. Russell

Introduction

The ionophore, monensin, has been used to modify ruminal fermentations since the 1970s, and this antibiotic decreases methane and ammonia losses. Monensin is most active against gram-positive bacteria and these species produce large amounts of hydrogen, a precursor of methane and ammonia, an end product of protein degradation. Extrapolation of this mode of action back to the rumen has, however, been stymied by difficulties in enumeration. In vitro, ruminal bacteria are surrounded by capsular material, and virtually all of them stain gram-negative or gram-variable.

Monensin is a metal/proton antiporter that causes potassium depletion, and this characteristic has been used as an index of monensin sensitivity. Some gram-negative ruminal bacteria are initially sensitive to monensin and must be adapted before they can grow. *Prevotella bryantii* is a gram-negative ruminal bacterium that can become highly resistant to monensin, but preliminary experiments indicated that it was initially monensin-sensitive. The following experiments sought to define whether this change was a simple adaptation of all cells or a selection of particular cells.

Methods

Prevotella bryantii B 4 was grown anaerobically in batch and continuous culture with glucose as an energy source. Cells were centrifuged through silicone oil, and cell pellets were digested at room temperature for 24 h in 3 N HNO_3. Potassium was determined with a flame photometer. Cells were extracted with perchloric acid and the supernatant fluid was assayed for ATP using the firefly luciferase method. Potassium depletion was estimated from the decrease in intracellular potassium as compared to controls (no monensin). Washed cells (160 µg protein/ml) were incubated in sodium phosphate containing 5 µM ^{14}C-labeled monensin. Cell pellets were resuspended in 10 ml of

scintillation fluid and counted. Wild-type and monensin-selected cultures were serially diluted in the basal medium containing or lacking monensin (10 µM). The most probable number was estimated from replicate (n = 5) dilutions. Wild-type and monensin-selected cultures were also serially diluted in molten basal agar medium containing or lacking monensin. Isolated colonies (n = 11) were picked. Cell pellets were resuspended in TRIS buffer and agglutination was monitored microscopically or via sedimentation after low speed centrifugation. Lipopolysaccharides were extracted without phenol and the aqueous phase above the phenol was removed, dialyzed and lyophilized to dryness. Lipopolysaccharide extracts were separated by thin layer chromatography. *Micrococcus luteus* cells were resuspended in TRIS buffer, lysozyme was added and lysis was estimated from the decrease in optical. Lipopolysaccharide of *P. bryantii* was added to estimate lysozyme inhibition. Alkaline phosphatase was determined with p-nitrophenyl phosphate.

Results and Discussion

Prevotella bryantii cultures treated with monensin grew more slowly, but only if monensin was greater than 1 µM. Cultures that were repeatedly transferred (8 transfers or 25 doublings) with monensin always grew rapidly, even at 10 µM monesin. The amount of monensin needed to facilitate half-maximal potassium depletion (K_d) from monensin-selected cells was 16-fold greater than "unadapted" wild-type cultures (3200 versus 200 nM). Cells taken from continuous culture had a K_d of 100 nM, and these inocula could not grow in batch culture when monensin was greater than 300 nM. Continuous cultures treated with monensin nearly washed out, but the surviving cells had a K_d of 1300 nM. When wild-type cells were transferred in batch culture with 10 µM monensin, K_d did not reach its maximum value (3200 nM) until 8 transfers (25 doublings). K_d declined when monensin was removed, and it took 8 transfers to reach the control value (200 nM). The MPN of wild-type cells was 1000-fold lower than monensin-selected cells, but calculations based on relative growth advantage and K_d indicated that the wild-type culture had 1 to 10% highly monensin-resistant cells. Cell pellets of wild-type cultures were more difficult to disperse than monensin-selected cells, and water-soluble phenol extracts of monensin-selected cells had 1.8-fold more anthrone reactive material than wild-type. Wild-type cultures that were washed in TRIS buffer (pH 8.0) released little alkaline phosphatase and were agglutinated by lysozyme. Monensin-selected cultures leaked 9-fold more alkaline phosphatase and were not agglutinated by lysozyme. Wild-type colonies taken from high-dilution agar roll tubes retained the lysozyme agglutination phenotype even if transferred with monensin, and monensin-selected colonies were never agglutinated. These observations indicated that wild-type *P. bryantii* cultures had a sub-population with different outer membrane characteristics and increased monensin-resistance.

Newbold *et al*. (1989) indicated that *P. ruminicola* (now *P. albensis*) cultures that were selected with the ionophore, tetronasin, bound less ^{14}C labeled tetronasin than wild-type cultures, and they hypothesized that the tetronasin-selected cells had smaller outer membrane porins. Our results indicated that monensin-selected *P. bryantii* cultures bound as much ^{14}C monensin as wild-type cultures, but monensin-selected cell pellets were easier to disperse than wild-type cells. Because phenol extracts from monensin-selected cells had twice as much anthrone reactive material as wild-type cells, it appeared that monensin resistance was mediated via an accumulation of hydrophilic carbohydrate rather than a decrease in porin size.

Previous work with mixed ruminal bacteria taken directly from cattle indicated that monensin supplementation caused an almost immediate increase in K_d, and the K_d reached its maximum value in only two days. Because the change in K_d was very rapid, it appeared that the rumen already had a large population of monensin-resistant bacteria. Wild-type *P. bryantii* and mixed ruminal bacteria from cattle not consuming monensin had similar K_d values (200 versus 180 nM, respectively), and the K_d values after monensin selection were also comparable (3200 versus 1800 nM, respectively). Batch cultures of *P. bryantii* were routinely selected with 10 µM monensin, a value that exceeds the in vivo concentration, but it should be noted that continuous cultures were much more sensitive and could be inhibited by as little as 0.04 µM monensin. Based on these results, it is conceivable that monensin-dependent changes in the K_d of mixed ruminal bacteria could be at least partially due to a selection of gram-negative bacteria as well as an inhibition of gram-positive species.

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Alternative Schemes of Butyrate Production in *Butyrivibrio fibrisolvens* and Their Relationship to Lactate Production and Phylogeny

F. Diez-Gonzalez, D.R. Bond, E. Jennings and J.B. Russell

Introduction

The rumen is well buffered with bicarbonate, but the rate of fermentation can at times be so rapid that ruminal pH declines. Ruminal acidosis causes decreased food intake and even death of the animal. Because lactate is a 10-fold stronger acid than the volatile fatty acids, the growth of lactate-producing bacteria promotes ruminal acidosis (Hungate *et al*. 1952). Some strains of *Butyrivibrio fibrisolvens* can produce large amounts of lactate, but the role of this bacterium in ruminal acidosis was unclear.

Hungate (1966) proposed that non-lactate-producing *B. fibrisolvens* strains should be reclassified as *B. alactacidigens*, but subsequent taxonomic descriptions deleted this designation. Recent work based on 16S rRNA gene sequence analysis indicated that ruminal *B. fibrisolvens* strains clearly fell into two phylogenetic groups, but Willems *et al*. (1996) concluded that "phenotypic data that support division of the genus *Butyrivibrio* are not available."

Based on the observation that lactate-producing strains of *B. fibrisolvens* are generally acetate-stimulated, we hypothesized that they might produce butyrate via butyryl CoA/acetate CoA transferase rather than butyrate kinase. Because lactate utilization and acetate stimulation have sometimes been correlated with current understanding of phylogenetic differences, we also hypothesized that butyryl CoA/acetate CoA-transferase might be related to the phylogenetic placement of these organisms.

Methods

B. fibrisolvens strains were grown anaerobically at 39° C in batch and continuous cultures with glucose as an energy source. Growth rate was estimated from the increase in optical density. Cultures were harvested anaerobically by centrifugation, and cell pellets were washed twice in Tris buffer. Cells were disrupted by sonication. Lactate dehydrogenase activity was determined by measuring the disappearance of NADH. Butyryl-CoA/acetate CoA-transferase activity was measured by following the decrease in butyryl-CoA concentration at 233 nm. Acetate kinase and butyrate kinase were assayed by hydroxamate formation. Chromosomal DNA was purified from strain B835 using a FastDNA Spin kit and the gene for the 16S rRNA was amplified using conserved primers. The amplified fragment corresponded to positions 27 through 1492 of the *Escherichia coli* 16S rRNA gene. PCR products were cloned immediately after PCR using a TA cloning kit and clones were sequenced using two primers. The 16S rRNA from B835 was aligned with other known *Butyrivibrio* 16S rRNA sequences using the Clustal method and similarity with selected *Butyrivibrio* sequences was calculated using a similarity matrix. Glucose, formate, lactate, acetate, and butyrate in cell-free supernatants were analyzed by high-pressure liquid chromatography.

Results and Discussion

Butyrivibrio fibrisolvens strains D1 and A38 produced little lactate, but strain 49 converted as much as 75% of its glucose to lactate. Strain 49 had 10-fold more lactate dehydrogenase activity than strains D1 or A38, was activated by fructose 1,6-bisphosphate, and had a pH optimum of 6.25. A role for fructose 1,6-bisphosphate or pH regulation of lactate production in strain 49 was, however, contradicted by the observations that very low concentrations (< 0.2 mM) of fructose 1,6-bisphosphate gave maximal activity, and continuous cultures did not produce additional lactate when the pH was decreased. The lactate production of strain 49 was clearly inhibited by the presence of acetate in the growth medium. When strain 49 was supplemented with as little as 5 mM acetate, lactate production decreased dramatically, and most of the glucose was converted to butyrate.

Strain 49 did not possess butyrate kinase activity, but it had a butyryl CoA/acetate CoA transferase that converted butyryl CoA directly to butyrate, using acetate as an acceptor. The transferase had a low affinity for acetate (K_m of 5 mM), and this characteristic explained the acetate stimulation of growth and butyrate formation. Strains D1 and A38 had butyrate kinase but not butyryl CoA/acetate CoA-transferase, and it appeared that this difference could explain the lack of acetate stimulation and lactate production. Because relatives of strain 49 (Nor37, PI-7, VV1, and OB156, based on 16S rRNA sequence analysis) all had the same method of butyrate production, it appeared that butyryl CoA/acetate CoA-transferase might be a phylogenetic characteristic.

Comparison of previous 16S rRNA analyses and our enzymatic data indicated that *B. fibrisolvens* strains having butyryl CoA/acetate CoA-transferase activity formed a phylogenetic cluster, but our strain B835 was an exception. However, previous the DNA-DNA hybridizations, the cell wall structure analysis and our 16S rRNA sequencing indicated that B835 was similar to strain Nor37, a strain that was only distantly related to A38 and D1. Based on these comparisons there are probably two "B835" strains in current use, one that is similar to A38 and D1 and one that clusters with 49. Willems *et al*. (1996) concluded that "phenotypic data that support division of the genus *Butyrivibrio* along phylogenetic lines are not available," but this conclusion was at least in part based on strain B835.

Shane *et al*. (1969) proposed that *B*. *fibrisolvens* could be partitioned into those that "produced appreciable amounts of lactate" and those that "produced acetate but little or no lactate," but some strains were ambiguous in this phenotype. Because *B*. *fibrisolvens* strains appear to utilize either butyryl CoA/acetate CoA transferase or butyrate kinase, but not both, it is conceivable that these pathways of butyrate production provide a connection between the phylogeny and physiology of these bacteria. Because *B*. *fibrisolvens* strains that were capable of producing lactate never did so when acetate was present (> 5 mM), it is unlikely that this bacterium would ever produce lactate in the rumen.

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Accounting for the Effects of a Ruminal Nitrogen Deficiency Within the Structure of the Cornell Net Carbohydrate and Protein Systems (CNCPS)

L.O. Tedeschi, D.G. Fox and J.B. Russell

The rumen typically operates as an energy-limited system, but diets can be so low in degradable crude protein that microbial growth is limited by N. Ruminal bacteria respond differently to N sources and N-limitation. Cellulolytic ruminal bacteria need ammonia as a nitrogen source, have little capacity to utilize amino N, are unable to ferment fiber when ammonia is depleted and do not produce ammonia from amino nitrogen. Some hemicellulose-digesting ruminal bacteria are stimulated by amino N, but even these species are primarily dependent on ammonia as a nitrogen source. Many non-fiber digesting ruminal bacteria prefer amino N over ammonia, and some can even produce ammonia from amino N sources.

The CNCPS uses rates of carbohydrate fermentation to estimate microbial growth in the rumen. Growth yields are adjusted to accommodate maintenance energy expenditures, peptide availability and pH, but the CNCPS does not have a provision for N-limitation per se. Ruminal N-limitation can decrease microbial flow (g bacteria/d) from the rumen, depress fiber fermentation and reduce DMI, but the CNCPS did not have equations to accommodate these effects. Most systems of ration formulation for cattle acknowledge the importance of supplying adequate nitrogen as well as energy, and the French system (INRA, 1989) uses either ruminally degraded nitrogen or ruminally available energy to predict microbial growth (depending on which one is the first limiting nutrient). However, not even the French system has a systematic method of discounting ruminal activity, microbial growth and DMI when ruminal N is depleted.

Our objectives were (1) to devise equations that could quantify the impact of N-limitation on fiber-digesting (FC) and non-fiber-digesting (NFC) bacteria, (2) use the equations to discount microbial protein production, fiber digestion, and DMI when N is limiting and (3) validate these CNCPS adjustments with experimental data with cattle responses to added dietary N.

Methods

Statistical measures were the regression r^2, mean standard error, slope and intercept confidence interval, and the residual plot. Residual plots were analyzed for outliers and systematic bias. Another test of model adequacy involved determining the proportion of deviation that lie within acceptable limits. Our limits were established as –.1 and .1 kg/d for ADG and –.5 and .5 kg/d for DMI comparisons. All the statistical analyses were performed using SAS. Regression parameters were estimated by PROC REG. The regression through the origin was obtained using NOINT option in PROC REG. The statistical comparison between observed and predicted values was performed using the two-sample T-test. The break point analysis consisted of the inclusion of extreme points and regressing them until the slope was not different from zero ($P > .05$), then the remaining points were used to develop the regression. The X value at the break point was calculated using the mean of the plateau as a Y-variate and solving for X. In order to evaluate the influence of ruminal N deficiency on microbial growth and consequently DMI depression, a multiple regression of predicted DMI and FCRedRatio values on observed DMI was evaluated.

Results and Discussion

The CNCPS prediction of fiber digestion and microbial mass production from ruminally degraded carbohydrate has been adjusted to accommodate a ruminal N deficiency. The steps for the adjustment are 1) the ruminal available peptide and ammonia pools are used to determine the N-allowable microbial growth, 2) this value is subtracted from the energy-allowable microbial growth to obtain the reduction in microbial mass, 3) this mass reduction is allocated between pools of bacteria digesting fiber (FC) and nonfiber (NFC) carbohydrate according to their original proportions in the energy-allowable microbial growth, 4) the reduction in fermented FC is computed as the FC bacterial mass reduction divided by its yield (g bacteria/g FC digested), and 5) this reduction is added to the FC fraction escaping the rumen. Five published studies had information that allowed us to evaluate the response of animals to added dietary N. These evaluations compared observed and CNCPS predicted average daily gain (ADG) with and without this adjustment. The adjustment decreased the CNCPS overprediction of ADG from 19.2 to 4.7%, mean bias declined from .16 to .04 kg/d, and the r^2 of the regression between observed and metabolizable energy (ME) or metabolizable protein (MP)-allowable ADG was increased from .83 to .88 with the adjustment. When the observed dry matter intake (DMI) was regressed against CNCPS predicted DMI with an adjustment for reduction in cell wall digestibility, the r^2 was increased from .77 to .88. These results indicated the adjustment for ruminal nitrogen deficiency increased the accuracy of the

CNCPS model in evaluating diets of growing animals when degraded N is deficient.

The N-limitation adjustment reduced the overpredictions (bacterial flow, fiber digestion, and DMI) of the 4.0 CNCPS, but they did not completely eliminate the bias. The ruminal N deficiency adjustment assumed that all other required nutrients for microbial growth were adequate, and this assumption may not always be valid. Ruminal cellulolytic bacteria require branched chain acids (isovaleric, isobutyric, and 2-methylbutyric) as well as N, and these acids are derived from the fermentation of branched chain amino acids (leucine, valine and isoleucine).

Branched chain acid production can be predicted from the fermentation of amino acids in the CNCPS, but at least some NFC bacteria can utilize branched chain acids. The question then becomes, what fraction of the ruminally available branched chain acids can be used by FC bacteria? Work is currently underway to modify the CNCPS to accommodate branched chain acid limitation.

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Accessibility to Potentially Degradable Cell Walls Limits Forage Degradability

H.G. Jung, M.A. Jorgensen, J.G. Linn and F.M. Engels

Introduction

While lignin concentration has repeatedly been shown to be negatively related to cell-wall degradability of forages, this relationship has more recently been found only to be true if the forages examined span a range of maturities. When the forages investigated are of similar maturity, then lignin is a poor predictor of cell-wall degradability. This observation calls into question the commonly assumed role of cell-wall chemistry in determining degradability of forages. Wilson and co-workers have proposed an alternative to the cell-wall chemistry model for limiting forage degradation. Based on microscopic observations of forage tissue degradation in the rumen, this alternate model proposes that while chemical structure of the cell wall does impact degradability, the dominant factor controlling degradability is the limited accessibility by rumen microorganisms to potentially degradable wall surfaces on the cell lumen side of the wall. The presence of a non-degradable, lignified middle lamella/primary wall acts as an impenetrable barrier to rumen microorganisms for the movement from one plant cell lumen to another. As a result, potentially degradable cell walls are protected from degradation by this impenetrable barrier. We have conducted a study to examine this alternative model for control of cell-wall degradability that used particle size reduction to eliminate these proposed impenetrable barriers by rupturing all forage cells and exposing their lumenal surfaces to microbial degradation.

Materials and Methods

Mature stem samples of corn (n = 16) and alfalfa (n = 18), harvested at the same maturity stage for each forage species, were selected from other studies to provide a wide range of cell-wall chemical compositions and degradabilities. These samples were initially ground through a 1-mm screen and then a portion of each sample was ball-milled. The 1-mm screen particles were of sufficient size that blocks of unruptured and potentially inaccessible cells remained, whereas the ball-milled samples were of such small particle size that almost every cell should have been disrupted. Microscopy and sieving were used to verify these assumptions. All forage samples were then analyzed for cell-wall concentration and composition (including neutral sugars, uronics, Klason lignin, monolignol units, and esterified and etherified hydroxycinnamates), and *in vitro* rumen degradability after 16- and 96-h fermentations. The impact of the accessibility model was assessed by comparing the degradation of cell-wall polysaccharides between the 1-mm ground and ball-milled samples.

Results and Discussion

A wide diversity in cell-wall composition and degradabilities was found among both the corn and alfalfa stem samples (Table 1). Ball-milling the forages did not significantly alter their cell-wall composition compared to grinding through a 1-mm screen. When the forage samples were incubated with rumen microorganisms, degradability of cell-wall polysaccharides was dramatically increased by ball-milling after both incubation periods (Figure 1). This result was expected from the accessibility model. There were differences among the sugar residues comprising the wall polysaccharides with regard to degree of increased degradability due to particle size reduction (data not shown). In the case of alfalfa, degradability of the sugars abundant in pectins did not markedly respond to decreased particle size. This observation is consistent with presence of pectins in nonlignified cells that are rapidly and completely degraded. For both forages, degradability of glucose and xylose residues from wall polysaccharides were greatly increased by particle size reduction through ball-milling. Glucose and xylose are predominantly components of cellulose and xylans, respectively, and these two polysaccharides are most abundant in thick secondary walls of tissues which are surrounded by lignified middle lamella/primary wall barriers.

Conclusions

Our results support the accessibility model for limitations to forage degradation. When forage samples were reduced in particle size to the level of individual disrupted cells, degradability of the cell wall was greatly increased. Cell-wall chemical composition (data not shown) was poorly related to degradability for either large or small particles. It appears modification of forage cell-wall chemical structure will be of limited benefit to improving forage utilization by ruminants unless the lignified middle lamella/primary wall barrier is modified to allow greater microbial access among adjoining plant cells.

Table 1. Range in data for cell-wall concentration and composition, and total cell-wall polysaccharide *in vitro* ruminal degradability of corn (n = 16) and alfalfa (n = 18) stems ground through a 1-mm screen in a cyclone-type mill or ball-milled.

	Corn		**Alfalfa**	
Trait[1]	Cyclone	Ball-milled	Cyclone	Ball-milled
	- - - - - - - - - - - - g kg^{-1} organic matter - - - - - - - - - - - - - - - - - -			
Cell wall	614 - 899	607 - 843	610 – 685	571 – 768
	- - - - - - - - - - - - - - g kg^{-1} cell wall - - - - - - - - - - - - - - - - -			
Glc	406 - 461	405 - 468	425 – 477	397 – 467
Xyl 231 - 269	199 - 271	114 – 135	111 – 202	
Ara 19 - 29	20 - 30	32 – 43	32 – 44	
Gal	7 - 12	7 - 19	27 – 35	20 – 39
Uronics	31 - 47	31 - 46	85 - 136	102 – 146
Klason lignin	169 - 232	171 - 231	188 - 214	154 – 237
p-Coumarate				
esters	15.54 - 39.97	14.43 - 35.23	0.08 - 0.14	0.06 - 0.12
ethers	nd	0 - 5.78	0.11 - 0.39	0.08 - 0.34
Ferulate				
esters	4.14 - 7.87	4.12 - 7.83	0.08 - 0.15	0.06 - 0.12
ethers	0 - 1.66	0 - 2.33	0 - 0.08	0.03 - 0.13
- - - - - - - - - - - - - - mol syringaldehyde mol^{-1} vanillin - - - - - - - - - - - - - -				
S/G ratio	0.93 - 2.00	0.86 - 1.92	0.40 - 0.61	0.36 - 0.59
	- - - - - - - - - - - - % - - - - - - - - - - - - -			
Total polysaccharide degradability				
16-h	11.2 – 34.2	20.1 – 46.9	30.4 – 45.9	58.7 – 86.5
96-h	37.7 – 60.2	69.7 – 90.5	46.0 – 64.5	71.7 – 81.3

[1]Glc, glucose; Xyl, xylose; Ara, arabinose; Gal, galactose; Man, mannose; Rha, rhamnose; Fuc, fucose; S/G ratio; syringyl-to-guaiacyl monolignol ratio.

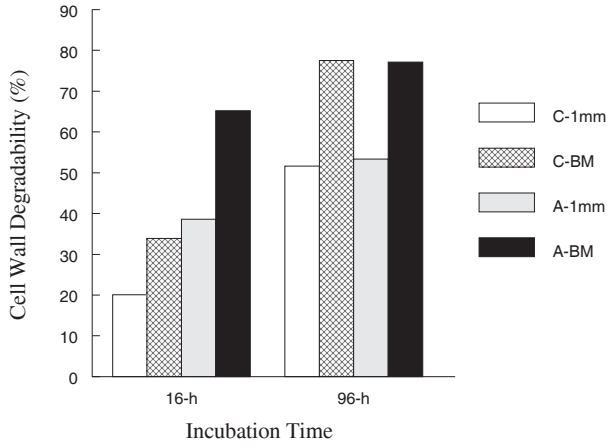

Figure 1. *In vitro* cell-wall degradability of corn (C) and alfalfa (A) stems that had been ground through a 1-mm screen (1mm) or ball-milled (BM).

Rate and Extent of Cell-Wall Degradation in Alfalfa Stem Tissues

H.G. Jung and F.M. Engels

Introduction

Because retention time of digesta in the rumen is limited in high-producing dairy cows due to large amounts of feed intake, the rate of cell-wall degradation is critical to determining how much digestible energy dairy cattle can extract from forages. Based on microscopic measurements of cell-wall thickness and degradation rates of mechanically isolated plant tissues, Wilson and Mertens calculated that the maximum potential rate of cell-wall degradation is approximately 0.02 mm h^{-1} for nonlignified mesophyll tissue in grasses. In addition, they predicted that < 40% of the wall could be degraded in 48 h of fermentation for thick-walled (~2.4 mm) grass sclerenchyma cells, if this normally lignified tissue were degraded at the same rate as thin-walled, nonlignified mesophyll tissue. Because legume cell walls are generally considered to be more rapidly but less extensively degraded then grasses, we examined the rate and extent of cell-wall degradation of alfalfa stem tissues.

Materials and Methods

The seventh internode of alfalfa stems was harvested after 4 wk of regrowth in 1996 and 1997. The internodes were preserved in 50% (vol/vol) ethanol:water. A series of 100-mm thick mirror section pairs were prepared from each stem internode and mounted on slides. One member of each mirror section pair was retained as a non-fermented control and the other mirror section member of each pair was assigned to a randomly chosen fermentation interval. The alfalfa stem sections were fermented for 2, 4, 8, 16, 24, 48, 72, or 96 h with rumen fluid. Slides were removed from the fermentation vessels at the specified times and stored in 50% (vol/vol) glycerol:water until examined microscopically. Degradation of epidermis, collenchyma, chlorenchyma, primary phloem, cambium, xylem vessels and fibers, protoxylem, protoxylem parenchyma, and pith parenchyma tissues was visually appraised by light microscopy (LM). Each fermented section was compared to its non-fermented control. Cell-wall thickness was measured on selected control and fermented section pairs by scanning electron microscopy (SEM).

Results and Discussion

The nonlignified tissues (epidermis, collenchyma, cambium, and protoxylem parenchyma) were very rapidly degraded, with complete degradation being observed after only 8 h of fermentation. This is illustrated in Figure 1 for some of these tissues. Primary phloem is a more complex tissue, with a lignified ring structure on the lumen side of the primary wall and a very thick nonlignified secondary wall. The secondary wall of primary phloem was completed degradable; however, this process required 24 h to complete

(Figure 1). Pith parenchyma was completely and rapidly degradable if nonlignified, but if the tissue had lignified then no degradation was observed by LM. The xylem fibers and vessels, and protoxylem were always lignified and appeared nondegradable by LM. Small amounts of wall thinning were measured for these apparently nondegradable, lignified tissues when examined under SEM. Wall thickness of nonlignified control tissues ranged from 0.29 (protoxylem parenchyma) to 1.70 (secondary wall of primary phloem) µm. Rates of degradation for these nonlignified tissues ranged from 0.04 (protoxylem parenchyma) to 0.11 (collenchyma) µm h^{-1}. Rate of wall degradation was not correlated with wall thickness of the tissue. The most rapidly degradable tissue (collenchyma) had thick cell walls that stained intensely for acidic polysaccharides such as pectins, whereas the more slowly degradable thick secondary wall of primary phloem did not stain for pectins but responded to polarized light indicating the presence of large amounts of cellulose. Pectin and cellulose are known to be rapidly and slowly degraded, respectively. For the lignified tissues, maximal extent of degradation were achieved within 24 h and these extents ranged from 9.1% (xylem fiber primary plus secondary wall) to 65.5% (additional secondary wall layer of xylem fibers). Wall thickness of tissues was not related to extent of degradation observed.

Conclusion

Clearly alfalfa stem tissues exhibit a range of cell-wall thickness and degradation kinetics. Degradation of nonlignified alfalfa stem tissues were 2 to 5 times faster than nonlignified grass mesophyll tissue. Unlike lignified grass sclerenchyma which can be extensively degraded if fermentation time is long, lignified alfalfa tissues reach their maximal extent of degradation relatively quickly and extent of degradation is less than observed for lignified grass tissues. Increasing the proportion of nonlignified tissues in alfalfa stems would increase both the rate and extent of degradation of this crop, improving its value as an energy source for high-producing dairy cows.

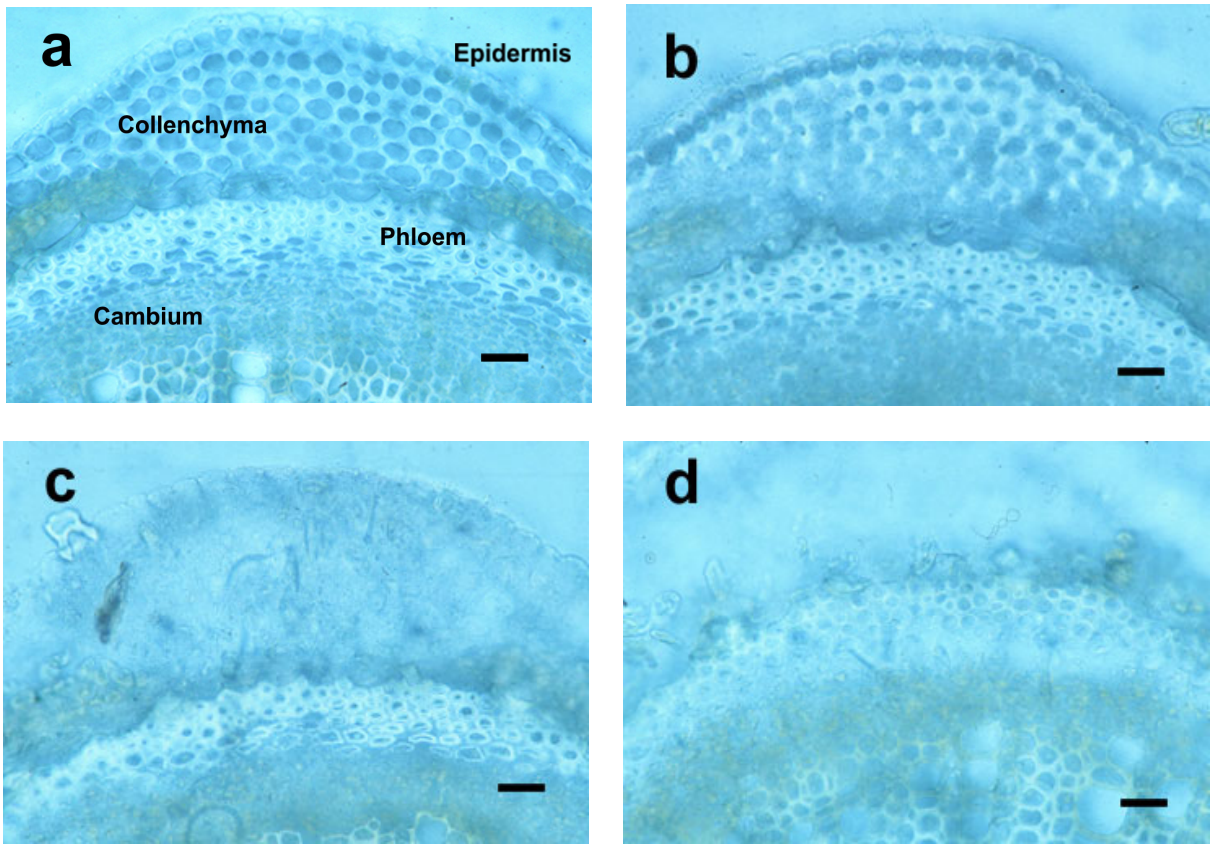

Figure 1. Degradation of epidermis, collenchyma, primary phloem, and cambium tissues after 0 (a), 4 (b), 8 (c), or 24 (d) h of *in vitro* fermentation by rumen microorganisms. The bar represents a length of 10 µm.

A Re-Examination of the Effect of pH on In Vitro Cellulose Digestion Kinetics

P.J. Weimer, F. Mouriño and R. Akarrawongsa

Introduction

Ruminal pH has long been recognized as one of the key environmental factors affecting the digestion of forage fiber. Pure cultures of the predominant ruminal cellulolytic bacteria cannot grow at pH values less than 6.0, and in situ experiments in which mineral acids were infused into the rumen to maintain pH at less than 6 have resulted in a loss of cellulose-digesting capabilities, presumably due to washout of the cellulolytic population. Modern dairy practices in much of the developed world employ feeding of energy-rich, high-grain diets to enhance production, and the ruminal fermentation of these diets often results in pH values decreasing to values below 6.0 for much of the feeding cycle. Despite the recognition of the importance of pH in controlling fiber digestion, little quantitative data are available that relate ruminal pH to the kinetics of cell wall digestion, and most of these data have been obtained under conditions where pH was intentionally controlled to minimize pH changes during the fermentation. This study was undertaken to determine the effect of pH on the rate of cellulose digestion by ruminal microorganisms when pH during the fermentation was allowed to change over a range observed in the rumen itself.

Materials and Methods

In vitro digestions were conducted in sealed 158 mL serum vials that contained a CO_2 gas phase, 250 mg of Sigmacell 50 microcrystalline cellulose, and 20 mL of buffer plus ruminal inoculum. The buffer was a Goering/Van Soest buffer adjusted to different pH values (5.2 to 6.8) with solid citric acid. The ruminal inocula were prepared by rotary-blending of ruminal fluid from two cows (squeezed through four layers of cheesecloth) with an equal volume of buffer of the desired pH and squeezed ruminal solids (0.25 g from each cow per mL of filtrate); the blended material was squeezed through four layers of cheesecloth and collected under CO_2. A total of nine different cows were sampled over the course of several experimental runs.

Pre-reduced culture vials were inoculated with varying levels of ruminal inoculum (5 to 25% of the original inoculum concentration by volume), and were shaken at 150 rev/min in a 39°C room. At various times, pairs of vials were removed, chilled in ice, and the pH of the culture was measured. Control vials lacking cellulose were recovered according to the same time schedule to allow for correction of fiber carried over with the inoculum. Neutral detergent solution (40 mL) was added to the vials, and residual cellulose was determined by a modified neutral detergent method. Rate constants and lag times for cellulose digestion were determined by fitting the data to a single pool exponential model employing a single discrete lag. In some experiments, paired vials were removed from incubation after 48 h and were analyzed separately for VFA by HPLC.

To assess microbial adherence to cellulose, fermentations were conducted as above, but at four times higher cellulose concentration to ensure that the fermentation was not cellulose-limited. Cellulose-adherent cells were estimated from N in cell pellets collected after three successive centrifugations (150 x g), with intermediate washes with 0.9% NaCl.

Results and Discussion

The first-order rate constant of cellulose digestion (*k)* was not affected ($P > 0.05$) by inoculum concentrations greater than 10% by volume, but lag time before initiation of cellulose digestion increased somewhat with decreasing inoculum concentration. To permit comparisons of *k* across experiments having different sources of inocula, rate constants were normalized within each run to values averaged from 15% and 25% inoculum concentrations and an initial pH of 6.56; the mean value of *k* across experiments under these conditions was 0.106 h^{-1}.

Once the fermentation passed an initial lag period, cellulose digestion displayed first-order kinetics with respect to cellulose concentration, despite substantial decreases in culture pH. The pH range over which first-order kinetics was observed varied from 0.3 to 1.1 units, and averaged 0.6 units for all combinations of initial pH and inoculum size. Because the value of *k* in a given culture remained constant over a substantial pH range, pH *per se* was not a direct determinant of cellulose digestion rate. Instead, it appears that the rate of cellulose digestion is governed by the pH at which the fermentation is initiated. For example, the value of *k* at pH 6.02 in cultures whose initial pH was 6.56 was substantially great than the value of *k* in cultures whose initial pH was 6.02. A plot of normalized *k* versus initial pH (Fig. 1) was linear, and *k* displayed a relative decrease of 7.3 per cent per 0.1 unit decline in pH. The data reveal that substantial cellulose digestion rates can occur at pH values below 6.0 (the minimum pH for growth of these bacteria), and cellulose fermentation stopped only at pH $\leq$ 5.3.

A/P ratios declined from a maximum of 1.76 at pH 6.74, to a minimum of 0.82 at an initial pH of 5.73, in accord with the data of Russell, who suggested that these shifts were due to changes in the relative activities of microbial subpopulations having different fermentation end product profiles.Estimates of cellulose-adherent microorganisms revealed that cell N reached a maximum about 10 h after the end of the lag

period. At this time, culture pH had declined to approximately 5.4.

Based on these observations, the fermentation of cellulose can be described by the model depicted in Fig. 2. The ruminal bacteria that hydrolyze cellulose compete effectively for the products of cellulose hydrolysis at pH values > 6.0, their minimum growth pH. At lower pH values, cellulose hydrolysis continues, but the hydrolytic products are used preferentially by non-cellulolytic bacteria to reduce the overall A/P ratio, an undesirable effect that can lead to milk fat depression. Cellulose digestion does not cease until pH values near 5.3, when adherent cells either detach from the fiber, or lyse.

Conclusions

The rate of cellulose digestion by ruminal microorganisms is dependent upon the pH at which the fermentation is initiated, which in vivo should correspond to the pH immediately prior to feeding. Maintenance of a prefeeding pH near 6.8 is likely to maximize cellulose digestion. Cellulose digestion can occur at pH values as low as 5.3, although at reduced rates and with a less desirable mix of fermentation end products.

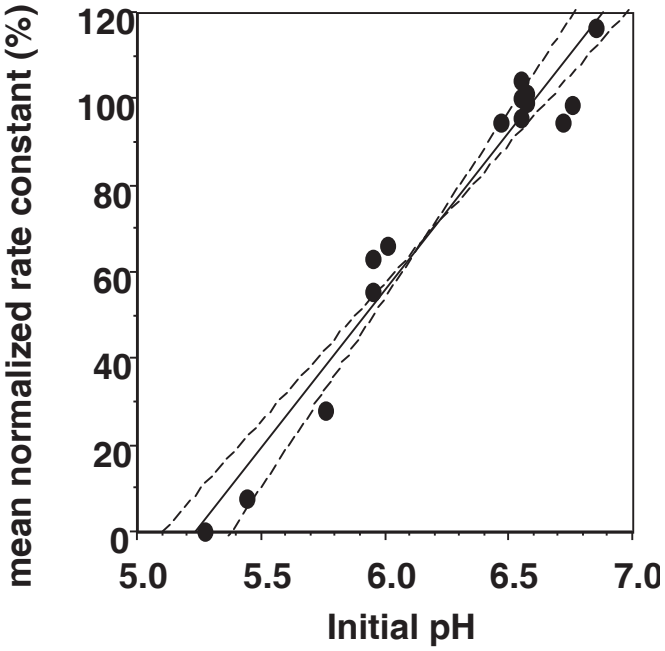

Figure 1. Effect of initial pH on the first-order rate constant of cellulose fermentation by mixed ruminal microflora. The rate constants were normalized to pH 6.56, at which the mean first order rate constant was 0.106 h^{-1}. Regression equation (solid line): normalized rate constant (%) = 72.82(pH) – 381.28; r^2=0.95. Broken lines represent 95% confidence intervals of the line slope.

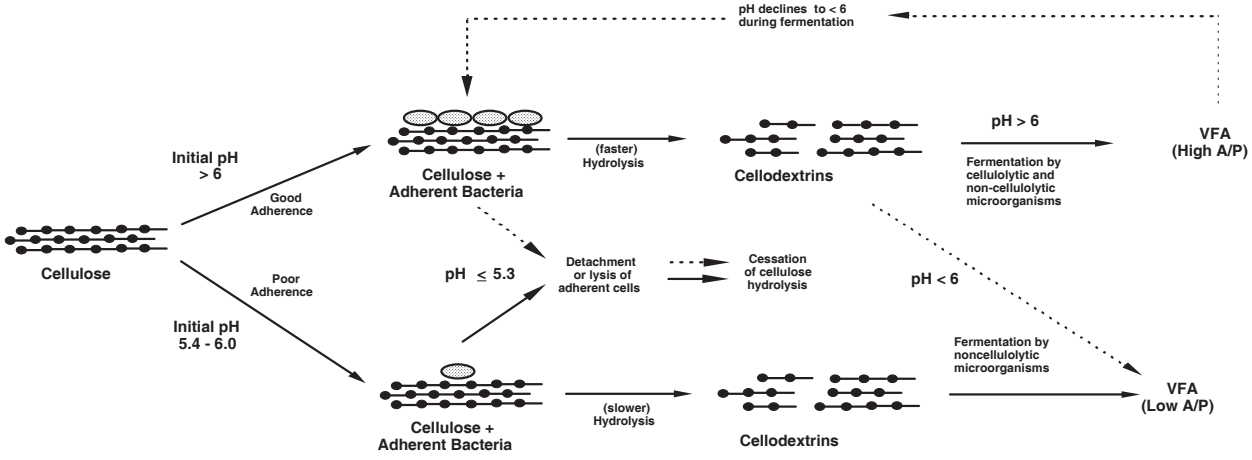

Figure 2. Model for cellulose digestion at high and low ruminal pH. Solid lines represent fermentations initiated at pH values above or below pH 6.0, as indicated. Broken lines represent cellulose fermentations at pH values < 6, after initiation of the fermentation at pH > 6. The relative sizes of cellulose, cellodextrins, and adherent bacteria are not to scale.

Milk Production, Reproductive Performance, and Fecal Excretion of Phosphorus by Dairy Cows Fed Three Amounts of Phosphorus

Z. Wu, L.D. Satter and R. Sojo

Introduction

Recent research suggests that dietary P can be reduced from high concentrations presently used to significantly lower concentrations without reducing animal performance. However, milk yield over short time periods may not be a good indicator of P status due to mobilization of P from bone. Thus, P status or adequacy needs to be evaluated over a long time frame. The objective of this study was to determine milk production and balance of P during a complete lactation in dairy cows fed different amounts of P.

Materials and Methods

Twenty-six multiparous Holstein cows were used in a 308-d lactation trial. Diets (Table 1) containing 0.31, 0.40, or 0.49% P (DM basis) were assigned to groups of 8, 9, and 9 cows at parturition based on their mature equivalent milk yield from the previous lactation. Molasses and beet pulp were included as diet ingredients because of their low P content, and this enabled formulation of a basal diet containing the low level of 0.31% P. Treatment diets containing 0.40 and 0.49% P were obtained by adding monosodium phosphate to the basal diet. The diets were fed as a TMR once daily for ad libitum consumption. Phosphorus balance was determined in the following 6 periods: wk 2, 4, 6, 8, 23 (22 to 24), and 40 (39 to 41) of lactation. The marker technique using Yb was employed to determine apparent digestibility of DM and P. A spot sample of urine also was taken. All samples of feces, milk, and urine were analyzed for P. From the concentration of P in milk and the average milk yield during the collection week, the amount of P secreted in milk per day was calculated. The amount of urinary P excreted was calculated from urinary P concentration multiplied by an estimated urine volume of 40 l/d, an average obtained from a separate study using the same herd and similar diets. Balance of P was calculated as the difference between P consumed and excreted or secreted in feces, urine, and milk.

Blood samples were taken daily from 2 d prepartum to 5 d postpartum, once during each of the six balance periods, and periodically during early to mid lactation, late lactation, and once during the dry period. Serum was analyzed for inorganic P concentration.

Results and Discussion

Phosphorus content of the diets averaged 0.31, 0.40, and 0.49% over the lactation (Table 1). Estimated Ca content was 0.65% for all diets.

Days to first estrus and first AI were shorter ($P < 0.05$) for the 0.31 and 0.49% P groups than for the 0.40% P group. Days open appeared to be shorter and more cows became pregnant at the first AI for the 0.31% P group. All cows were conceived by 206 DIM except one in each of the 0.40 and 0.49% P groups. More services ($P = 0.10$) were required per conception as dietary P was increased. While the number of animals used in this experiment is too small to draw reliable conclusions about reproduction, the data do suggest that reproductive performance does not have to suffer when dietary P is reduced to 0.31%.

Mean DMI over the lactation was similar ($P > 0.05$) among treatments (Table 2). Milk yield for the total lactation was not different either. Overall, all groups of cows milked well, averaging 11,050 kg for the 308-d lactation. The lactation curves were unusually flat for approximately 20 wk after peaking, reflecting the use of bST. It appeared, however, that the low P group began to separate from other treatment groups around wk 25 of lactation. On average, milk yield was 3.3 kg/d lower for the 0.31% P group than for the other two groups during wk 25 to 44 of lactation. The content of milk components did not differ ($P > 0.05$) among treatments. No differences between treatment groups were observed for body weight gain.

Results indicate that feeding 0.40 or 0.49% P throughout the lactation did not affect feed intake, milk production, or body condition of cows; reducing dietary P to 0.31% supported comparable milk production during approximately the first 2/3 of lactation, but appeared to decrease milk yield during the last 1/3 of lactation.

The concentration of inorganic P in blood serum was distinctly lower for the 0.31% P group than for the other two groups during the first 14 d postpartum. During the sampling periods after d 14, little or no difference in serum P concentration between treatment groups was evident. The lowest concentration measured in the present study was slightly below 5 mg/dl, marginally above the level (4 mg/dl) that is considered normal for lactating cows.

The P concentration of feces increased as dietary P content was increased, with the average concentrations from all weeks being 0.508, 0.728, and 0.898% on a DM basis for the 0.31, 0.40, and 0.49% P treatments, respectively. Assuming that the fecal output was the same for all treatments, the fecal P concentration averages suggest that decreasing dietary P from 0.49 to 0.40% reduced fecal P excretion by 23%. Apparent digestibility of P was similar for wk 2 to 40 for the 0.31% P treatment, and wk 2 to 8 for the 0.40% and 0.49% P treatments. The overall average for apparent digestibility of P during these weeks for the three treatments was 47%. With the exception of wk 4 for the 0.49% P treatment where apparent digestibility of P was 56.3%, it appeared that 47% apparent digestibility represented an upper limit.

Cows in the 0.31% P treatment were in negative P balance during the first 6 to 7 wk of lactation, and then hovered at near balance until the last sampling week when a positive balance was noted. This would seem to conflict with the lactation data which suggested that cows in the 0.31% P treatment began to reduce their milk production around wk 25 because of inadequate P intake. The positive P balance at wk 40 is surprising, unless one reasons that the cow at this stage of lactation and pregnancy is giving priority to supplying the fetus with P and repleneshing bone P in preparation for the next lactation.

Table 1. Ingredients and nutrient content of diets.

	Dietary phosphorus content (%)		
Item	0.31	0.40	0.49
Ingredient, %			
Alfalfa silage	30.0	30.0	30.0
Corn silage	20.0	20.0	20.0
High moisture ear corn	25.5	25.5	25.5
Soybeans, roasted	10.0	10.0	10.0
Blood meal	2.0	2.0	2.0
Molasses	5.0	5.0	5.0
Beet pulp	7.0	6.58	6.16
Salt, mineral and vit. mix	0.5	0.5	0.5
Monosodium phosphate	. . .	0.42	0.84
Chemical composition			
CP, %	17.8	17.8	17.8
ADF, %	23.8	23.7	23.6
NDF, %	31.4	31.2	31.0
P, %	0.31	0.40	0.49

Table 2. Performance of cows fed diets differing in phosphorus content.

	Treatment				*P*		
Item	0.31% P	0.40% P	0.49% P	SEM	L[1]	Q[1]	T x M[1]
DMI, kg/d	23.0	22.4	23.4	0.2	0.19	0.01	0.01
Milk, kg/308-d	10,790	11,226	11,134	607	0.69	0.72	. .
Milk, kg/d	35.0	36.5	36.2	0.7	0.27	0.32	0.01
3.5% FCM, kg/d	36.4	37.5	37.9	0.7	0.17	0.69	0.01
Milk fat, %	3.66	3.69	3.71	0.05	0.51	0.94	0.99
Milk fat, kg/d	1.333	1.344	1.348	0.028	0.71	0.92	0.01
Milk protein, %	3.14	3.07	3.11	0.03	0.57	0.16	0.01
Milk protein, kg/d	1.128	1.099	1.123	0.019	0.86	0.26	0.01

[1]Effect of linear, quadratic, and treatment by month interaction.

Milk Production and Reproductive Performance of Dairy Cows Fed Two Amounts of Phosphorus for Two Years

Z. Wu and L.D. Satter

Introduction

Several studies have recently suggested that P in lactation diets may be reduced from the current feeding level without affecting milk production, but more data from long-term studies are needed. The objective of this 2-yr study was to measure performance of dairy cows when fed a diet with no supplemental P or a diet supplemented with P to a level that is commonly used by dairy producers.

Materials and Methods

The experiment included two dietary P concentrations, one that was close to the NRC P recommendation (low), and one that was in excess of the NRC recommendation (high). The trial was carried out for 2 yr. Each year included confinement feeding during the first 2/3 of the lactation and grazing during the last 1/3 of the lactation. In yr 1, 42 cows (20 primiparous) were assigned randomly to treatments within parity at calving (n = 21, including 10 primiparous animals in each group). In yr 2, 14 cows (7 primiparous) from the low P group and 16 cows (8 primiparous) from the high P group continued with their treatments for another full lactation. Twelve (6 primiparous) new cows were added to the low P group and 11 (6 primiparous) to the high P group. Thus, a total of 53 Holstein cows were used in yr 2. Over the 2 years, 95 lactations (42 in yr 1 and 53 in yr 2; 32 from primiparous cows and 63 from multiparous cows) involving 63 cows were used. Thirty of the cows were used in both years, including 15 primiparous in yr 1.

Formulation of the TMR (Table 1), which was fed during confinement, was the same for both years, and no change was made during the lactation. The low P diet contained no supplemental P, while the high P diet was formulated by adding monosodium phosphate and dicalcium phosphate to the low P diet in place of high moisture ear corn. Grazing cows were supplemented with concentrate mixes. Formulation of the supplemental mixes (Table 1) was the same for both years. The mix fed to the low P cows contained no supplemental P while the mix fed to the high P cows contained dicalcium phosphate. The supplements were fed separately to each treatment group at a rate of 6.2 kg/d per cow in yr 1 and 7.9 kg/d per cow in yr 2 (DM basis). These amounts accounted for approximately 35 to 40% of total DMI.

Blood was sampled periodically, with sampling times falling within the following weeks of lactation: 1 to 5, 6 to 15, 16 to 25, 26 to 34, and 35 to 44 in yr 1, and 1 to 5, 6 to 12, 13 to 18, 19 to 25, 26 to 31, and 32 to 37 in yr 2. Blood samples were also taken during the dry period once in each year, on an average of 43 d (SD 18) in yr 1 and 28 d (SD 20) in yr 2 after dry-off.

Results and Discussion

Dietary P content was 0.38 and 0.48% of DM during confinement feeding for the low and high P diets for both years (Table 1). The corresponding amounts during grazing were 0.31 and 0.44%, calculated from intake of the supplements and estimated intake of forage (2 : 3).

The concentration of inorganic P in blood serum appeared slightly lower for cows fed the low P diet than for those fed the high P diet with means being 6.1 vs. 6.7 mg/dl (SE 0.3, $P = 0.11$) for yr 1 and 6.2 vs. 6.4 mg/dl (SE 0.2, $P = 0.13$) for yr 2. The concentrations appeared similar at the end of lactation. All concentrations (5.6 to 7.4 mg/dl) were within the normal range (4 to 8 mg/dl) typically seen in lactating cows. Blood serum concentrations during the dry period increased compared with the last measurement during lactation, but were similar between treatments, reflecting the fact that cows were fed the same diets during this period.

There was no consistent difference in reproductive performance between the P groups in either year, as measured in days to first estrus, days to first AI, days open, conception rates, pregnancy rates, and services per conception.

Averages of DMI during confinement feeding were similar between groups in both years (Table 2), indicating that feeding P at 0.38 or 0.48% of the diet had no effect on ad libitum feed intake. Milk yields for the entire lactation were not different between treatments in either year (Table 2), and at no time during lactation did the high P group appear to yield more milk than the low P group. Grazing contributed to the decline of the lactation curves during the latter part of the lactation. Mean milk fat percentage, fat yield, and 3.5% FCM yield did not differ between treatments in either year. Milk protein percentage was lower ($P = 0.06$) for low P than for high P in yr 1, but did not differ in yr 2. Milk protein yield did not differ between treatments in either year. The content of lactose, SNF, or SCC of milk was not different.

Conclusions

Cows in this two-year study fed diets with no supplemental P (0.38% dietary P during confinement feeding for approximately 2/3 of the lactation and 0.31% P during grazing for approximately 1/3 of the lactation) performed as well as cows receiving supplemental P (0.48% P during confinement feeding and 0.44% P during grazing). Blood

serum P concentrations were slightly lower, but within normal ranges when the low P diet was fed. Cows that received the low P diet in the second year performed similarly between treatments. Phosphorus at 0.38 to 0.40% of diet DM should be adequate for cows producing ~ 11400 kg/308 d.

Table 1. Ingredient and chemical composition of diets and supplements.

Item	Diets during confinement		Supplements during grazing[1]	
	Low P	High P	Low P	High P
	---------- % of diet DM----------			
Ingredient				
Alfalfa silage	30.0	30.0	. . .	. . .
Corn silage	20.0	20.0	11.0	11.0
High moisture ear corn	28.4	28.0	74.7	74.1
Soybean meal	8.0	8.0	. . .	. . .
Soybean, roasted	12.0	12.0	10.6	10.6
Monosodium phosphate	. . .	0.3	. . .	. . .
Calcium carbonate	1.1	1.0	2.8	1.7
Dicalcium phosphate	. . .	0.2	. . .	1.7
Salt, mineral and vit. Mix	0.5	0.5	0.7	0.7
Chemical composition				
Year 1				
CP, %	19.2	19.2	11.1	11.1
NDF, %	28.6	28.5	18.9	18.8
ADF, %	19.5	19.5	8.7	8.7
P, %	0.38	0.48	0.33	0.65
Year 2				
CP, %	19.7	19.6	12.1	12.1
NDF, %	28.3	28.3	16.5	16.4
ADF, %	19.8	19.8	7.4	7.4
P, %	0.38	0.48	0.33	0.65

[1]Supplements during grazing were offered at 6.2 kg/d per cow in yr 1 and 7.9 kg/d per cow in yr 2 to provide approximately 35 to 40% of total DMI.

Table 2. Lactation performance of cows fed diets containing low or high phosphorus for two years.

	Year 1				Year 2			
Item	Low P (n = 21)	High P (n = 21)	SEM	*P*	Low P (n = 26)	High P (n = 27)	SEM	*P*
DMI, kg/d	20.7	20.4	. .	. .	23.2	23.4	. .	. .
Milk, kg/308 d	9131	8860	283	0.50	9864	9898	340	0.94
3.5% FCM, kg/d	29.6	29.3	0.9	0.85	34.1	33.9	1.0	0.89
Milk fat, %	3.46	3.58	0.09	0.37	3.7	3.65	0.10	0.38
Milk fat, kg/d	1.02	1.03	0.03	0.85	1.2	1.20	0.04	0.68
Milk protein, %	3.05	3.16	0.04	0.06	3.14	3.14	0.04	0.96
Milk protein, kg/d	0.91	0.93	0.02	0.73	1.01	1.03	0.03	0.73

Feeding High Forage Diets to Lactating Dairy Cows

V.R. Moreira, H.S. Santos and L.D. Satter

Introduction

Under normal conditions it usually is profitable to feed as much grain or high-energy by-product feed as a cow can handle without getting acidosis or severe milk fat depression. Use of more highly digestible forages, however, may permit higher inclusion rates of forages in diets that can be healthier for the cow, and possibly more profitable. Brown midrib-3 corn silage is such a forage, as this variety has a reduced amount of lignin, and has higher digestibility.

This preliminary experiment was conducted to determine the effect of very high forage diets (75% of diet DM) on performance of midlactation cows. A limited amount of brown midrib corn silage was available from an earlier experiment, so this study was of necessity a very short-term trial.

Materials and Methods

Twenty-two primiparous and twenty-six multiparous cows averaging 138 days in milk and 38.1 kg milk/d were assigned to a completely randomized design, with 16 cows assigned to each of three treatments. Cows received bovine somatotropin. Cows were individually fed the treatment diets for 24 days.

The experimental diets are shown in Table 1. Two of the treatment diets had a high forage:concentrate ratio of 75:25 using brown midrib-3 (BMR) (Cargill full-time) or a blend of regular hybrids (RCSH) as the source of corn silage in the diets. The third treatment had a forage:concentrate ratio of 55:45, and the same blend of regular hybrids supplied the corn silage for this diet (RCS). All cows were fed a similar diet containing regular corn silage during a 14-day adaptation period prior to going on the treatment diets.

Table 1. Diet composition of the three treatments.

Ingredient, % of DM	BMR	RCSH	RCS
Corn Silage	40.0	40.0	29.6
Alfalfa Silage	35.0	35.0	25.9
High Moisture Shelled Corn	8.8	8.8	25.9
Roasted Soybeans	11.2	11.2	11.2
Soyplus	3.7	3.7	2.1
Soybean Meal (48% CP)	0.0	0.0	3.6
Mineral and Vitamin Mix	1.3	1.3	1.7
Dry Matter (%)	45.3	44.6	52.1
Crude Protein (%)	17.6	17.6	17.6
Neutral Detergent Fiber (%)	34.2	34.2	27.8
Acid Detergent Fiber (%)	25.2	26.6	20.7

Milk production was measured daily, and milk samples for milk composition analyses were obtained weekly. Feed offered and refused was recorded daily. Results were statistically analyzed using the mixed model procedure of SAS 7.0 (Proc Mixed) set for a completely randomized design.

Results and Discussion

Results are shown in Table 2. Dry matter intake was lowest for the RCSH treatment. Milk yield was greatest for the RCS treatment, followed by the BMR and RCSH treatments. The high forage treatments had much higher milk fat and lower milk protein than the moderate forage diet. Fat corrected milk production for the BMR and RCS treatments was similar.

Conclusion

Caution must be used in drawing conclusions from such a short-term experiment. Longer treatment periods might easily have resulted in greater differences in milk production between the high and moderate forage diets. As it was, however, cows fed the brown midrib corn silage handled the high forage diet quite well. Longer-term studies are needed to evaluate cow health and profitability implications of such high forage diets.

Table 2. Dry matter intake and milk production.

	BMR	RCSH	RCS	SEM	P<
DM intake (kg/d)	19.5[a]	17.6[b]	19.5[a]	0.25	0.0001
Milk yield (kg/d)	34.l[b]	32.l[c]	35.7[a]	0.35	0.001
Milk fat (%)	3.68[a]	3.75[a]	3.36[b]	0.08	0.006
Milk protein	2.95[b]	2.97[b]	3.13[a]	0.03	0.0002
Milk lactose	4.69[b]	4.70[b]	4.79[a]	0.03	0.01
3.5% FCM (kg/d)	34.7[a]	33.2[b]	34.4[ab]	0.66	0.11
Fat yield (kg/d)	1.24	1.20	1.18	0.03	NS
Protein yield (kg/d)	0.99[b]	0.95[b]	1.10[a]	0.02	0.0001
Lactose yield (kg/d)	1.58[b]	1.51[c]	1.70[a]	0.02	0.04
SNF yield (kg/d)	2.85[b]	2.73[c]	3.09[a]	0.04	0.04
SCC (,000)	465	356	289	147	NS

High Corn Silage Diets for Lactating Cows

V.R. Moreira, C.V. Cragnolino and L.D. Satter

Introduction

High corn silage yields relative to alfalfa contribute to lower cost per ton of DM for corn silage than for alfalfa. Inclusion of some alfalfa in an otherwise high corn silage diet is normally recommended. The purpose of this study was to compare the relative effectiveness of low quality (high fiber) alfalfa hay and two levels of high quality alfalfa silage in maintaining rumen environment and milk fat test when corn silage constituted 50 - 75% of the forage in diets having a forage:concentrate ratio of 50:50.

Methods and Procedures

Twenty-four Holstein cows averaging 132 days in milk were assigned to four treatments in a 4 x 4 Latin square design replicated 6 times. Four of the cows had ruminal cannulas, thus enabling sampling of ruminal digesta. Periods were 21 days, allowing 14 days for adaptation and 7 days for sample collection. The four treatments consisted of four forage combinations in diets containing 50:50 forage:concentrate (dry basis). The treatments were: all corn silage (CS), 75% corn silage and 25% low quality alfalfa hay (3/4 CS 1/4 AH), 75% corn silage and 25% alfalfa silage (3/4 CS 1/4 AS), or 50% corn silage and 50% alfalfa silage (1/2 CS 1/2 AS). The corn hybrid used was Dairyland Forecast 3000 (Dairyland Seeds). The crude protein, NDF and ADF (% of DM) were: 16.6, 57.5, and 49.8; 22.5, 41.1 and 36.1; 7.66, 41.2 and 25.9 for alfalfa hay, alfalfa silage and corn silage, respectively. Diet ingredients and chemical composition are shown in Table I.

Results and Discussion

Dry matter intake and milk production and composition are shown in Table 2. Dry matter intake as well as milk production were reduced on the 3/4 CS 1/4 AH treatment. The two treatments containing alfalfa silage supported more milk than did the CS treatment. Dry matter digestibility of the diets, using ytterbium as an external marker, was not different between diets, and averaged 67.5%. Concentrations of ruminal VFA are in Table 3. The AC:PR ratio was highest for the 1/2 CS 1/2 AS treatment, and was generally higher for the treatments containing alfalfa. Average ruminal pH did not differ between treatments, but the fluctuation, i.e., difference between the high and low pH, was greater for the CS treatment.

Conclusion

Satisfactory milk production levels can be achieved when corn silage is the only forage, but better performance is obtained with a mixture of corn silage and alfalfa silage. Feeding low quality alfalfa hay to provide fiber in a high corn silage diet is not recommended. The best performing forage mixture in this experiment was the 1/2 CS 1/2 AS mix.

Table 1. Diet ingredients and chemical composition.

	CS	3/4 CS 1/4 AH	3/4 CS 1/4 AS	1/2 CS 1/2 AS
Ingredient (% of DM)				
Alfalfa hay	- -	12.5	- -	- -
Corn silage	50.0	37.5	37.5	25.0
Alfalfa silage	—	—	12.5	25.0
Soybean hulls	2.9	2.9	2.9	2.9
High moisture shelled corn	21.2	24.1	26.3	30.7
Roasted soybeans	11.6	11.6	11.6	11.6
Soybean meal (48% CP)	10.8	8.1	5.9	1.8
Blood meal	0.9	0.9	0.9	0.9
Mineral, yeast and vitamin mix	2.6	2.4	2.4	2.1
Chemical analyses of diets				
DM (%)	59.1	64.8	58.6	58.3
CP (% DM)	17.8	17.7	17.4	17.5
NDF (% DM)	26.7	28.7	26.6	26.6
ADF (% DM)	16.1	19.0	17.2	18.4

Table 2. Dry matter intake and milk production and composition.

	Treatments					
	CS	3/4 CS 1/4 AH	3/4 CS 1/4 AS	1/2CS 1/2 AS	SEM	P <
DM Intake, kg/d	23.3[b]	21.4[c]	24.6[a]	24.2[a]	0.47	.01
Milk, kg/d	39.6[b]	39.1[b]	40.0[ab]	41.0[a]	0.75	.03
3.5% FCM, kg/d	35.9[b]	37.1[ab]	37.3[ab]	38.9[a]	1.05	.005
Milk Fat, %	3.05[b]	3.13[ab]	3.22[a]	3.10[ab]	0.15	.07
Milk Protein, %	3.32[a]	3.28[ab]	3.29[a]	3.20[b]	0.07	.04
Lactose, %	4.77	4.74	4.80	4.70	0.06	NS
Fat, kg/d	1.17[b]	1.25[a]	1.26[a]	1.26[a]	0.06	.05
Protein, kg/d	1.28	1.31	1.30	1.30	0.03	NS

Table 3. Concentrations of ruminal volatile fatty acids..

	Treatments					
	CS	3/4 CS 1/4 AH	3/4 CS 1/4 AS	1/2CS 1/2 AS	SEM	P <
Acetate (mM)	67.3[b]	73.5[ab]	70.6[ab]	76.3[a]	1.97	0.02
Propionate (mM)	42.6[a]	34.9[ab]	32.9[ab]	30.0[b]	4.21	0.02
Butyrate (mM)	13.4[b]	14.2[ab]	14.4[ab]	16.6[a]	0.73	0.02
Valerate (mM)	3.72	2.99	2.75	3.33	0.79	NS
Isovalerate (mM)	2.67	2.55	2.46	2.46	0.24	NS
Isobutyrate (mM)	1.38	1.23	1.21	1.27	0.09	NS
Total VFA (mM)	131	129	124	130	4.45	NS
A:P	1.63[b]	2.44[b]	2.40[b]	2.89[a]	0.34	0.01

Phosphorus Content of Feeds and Feces on Wisconsin Dairy Farms

J.M. Powell, D. Jackson-Smith and L.D. Satter

Introduction

Many dairies in the U.S. continue to be land-based; that is, they produce most of their feed and use their land base to recycle manure nutrients through crops. To remain economically viable, many dairies are increasing herd size and importing more and more feed. The amounts of manure nutrients often exceed field crop requirements. This can lead to disposal rather than an agronomic use of manure and subsequent nutrient build-up and losses to the environment. Excessive soil nutrient accumulation, runoff and the pollution of surface and ground waters are the most pressing environmental challenges facing these farming systems. Legislation aimed at controlling farmer practices that potentially pollute surface and ground waters is becoming stricter. The survival of many dairies will depend on farmers' ability to comply with increasingly strict environmental regulations, especially those associated with the application of manure P to cropland. Recent research at the DFRC has shown that dietary P can be reduced by 25 to 30%, which reduces manure P by a greater percentage, without sacrificing milk production or quality. (Satter and Wu, 1999). This survey was undertaken to determine the P feeding strategies of dairy producers in Wisconsin and identify the potential for reducing dietary P levels under farmer conditions.

Methods

A total of 98 dairy farms were randomly selected across the top 17 dairy counties in Wisconsin. These counties represent over half of all the herds, cows and milk produced in the state. On-farm interviews were conducted to gather information on (1) general farm characteristics including scale of operation, breeds, nature of milking and housing facilities, milk yield, the use of various production technologies and management practices, (2) dairy herd feeding practices including how and what cows are fed, the importance of different factors and sources of information in determining rations, the use of hired consultants and forage testing services, and details regarding the feeding of P in dairy herd diets, (3) the farm's cropping activities including acres operated and crops grown, and (4) information about farms operator demographics and their plans for the future. This report summarizes results of P feeding practices and relationships between dietary P levels and the P content of feces. Farm operators were first asked if the lactating herd was subdivided into different feeding groups. The types and amounts of feed being fed on the day of the interview were recorded for each feeding group. Each feed component was sampled and analyzed for dry matter (DM) and total P content. Freshly deposited feces were sampled from the barn floor and analyzed for total and water-soluble P. Dietary P levels were calculated by the proportionate combination of feed components DM and associated P content.

Results and Discussion

Of the 98 surveyed farms, 93 had complete data sets on apparent feed dry matter and P intake. Of these 93 farms, 47 feed all lactating cows the same and 46 divide the lactating herd into 2 or 3 separate feeding groups. Approximately 70% of the surveyed farms said they were self sufficient in forage (alfalfa and corn silage) and grain (corn and oats) production. On these farms, approximately 90% of the apparent DM and 72% of the P intake is derived from these homegrown feeds (Table 1). Most of the remaining DM and P that is fed to the dairy herd are imported in the form of protein supplements, mineral mix and soybean meal.

The average P contents of forages and grain fed to dairy cows in Wisconsin are similar to national averages (Table 2). These P concentrations, especially for alfalfa, are higher than the NRC book values that are used to formulate dairy diets (Berger, 1995). Variability in P content of alfalfa and corn silage appears to be higher in the Wisconsin than the national sample.

Most dairy producers feed P well in excess of the NRC recommended amount. Approximately 20% of the surveyed farms feed less than the NRC recommendations (i.e.19 farms have dietary P levels and associated milk responses to the left of NRC recommendation). These results indicate that NRC recommends excessive dietary P levels, and corroborate recent experimental results at the DFRC (Satter and Wu, 1999) that lower amounts of P could be fed without reductions in milk production.

For all herds (n = 93), there was a relatively poor relationship ($R^2 = 0.33$) between fecal P and feed P concentrations. This relationship improved (R^2 of 0.55) for herds fed as a single group (Fig. 2). This difference in relationships implies difficulty in representative, whole herd sampling of feces when the herd is divided into multiple feeding groups. For herds fed similarly, it appears that fecal P may be used to estimate levels of dietary P.

Conclusions

Most dairy farms in Wisconsin are self-sufficient in forage and grain production. Lactating dairy cows derive 90% of their DM intake and 78% of their P requirement from these diet components. The P content of forages and grains is higher than the NRC tabular values that are widely used in formulating dairy diets. This is particularly true for alfalfa, a major component of dairy rations. Using NRC tabular values for P may contribute to overfeeding of P. Well over

half of the dairy cows appear to be fed P in excess of what is needed for the milk production levels that are attained. The P content of feces may provide a good indicator of the P content of the diet.

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Table 1. Relative amounts of forage and grain fed on farms (n = 64) that are self-sufficient in forage and grain production.

	Mean	Std. Dev.	Minimum	Maximum
Forage				
DM fed (kg/cow/day)	12.2	3.4	4.8	22.2
% of total DM	60	13	23	94
P fed (g/day)	34	13	13	66
% of total P	44	16	13	90
Forage + grain				
DM fed (kg/cow/day)	18.4	3.8	6.4	32.0
% of total DM	90	9	69	100
P fed (g/day)	57	20	18	139
% of total P	72	19	37	100

Table 2. Comparison of the feed P contents in Wisconsin and in the U.S.

Feed	Wisconsin Survey			U.S. Survey (Berger, 1995)		
	samples	mean	sd	samples	mean	sd
		- - P (g/kg) - -			- - - P (g/kg) - - -	
Alfalfa	170	2.8	0.74	4096	3.0	0.60
Corn silage	79	2.4	0.80	8197	2.3	0.60
Corn grain	63	3.1	0.47	912	3.2	0.70
Soybean meal	12	6.4	0.71	148	7.2	2.80

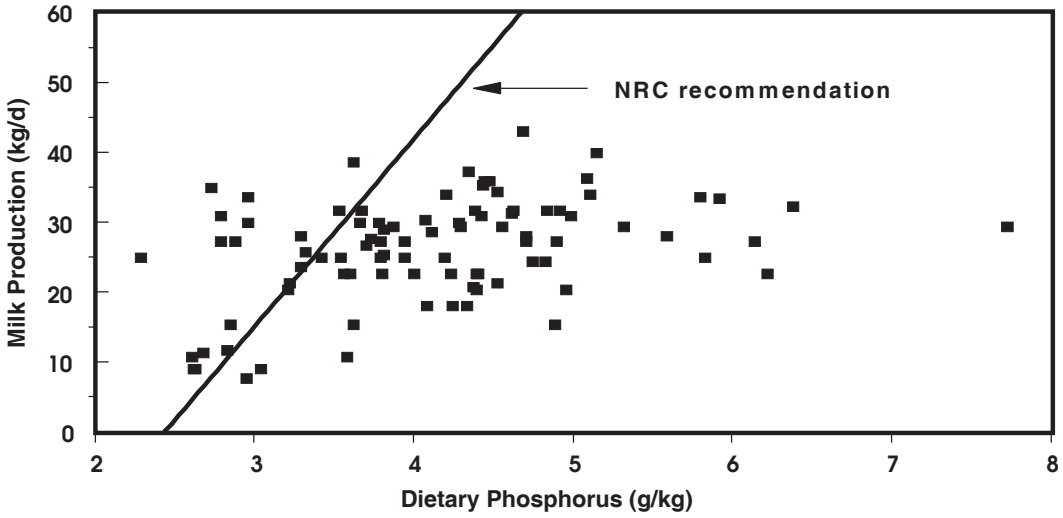

Figure 1. Relationships between diet P content and milk production, 93 dairy farms in Wisconsin

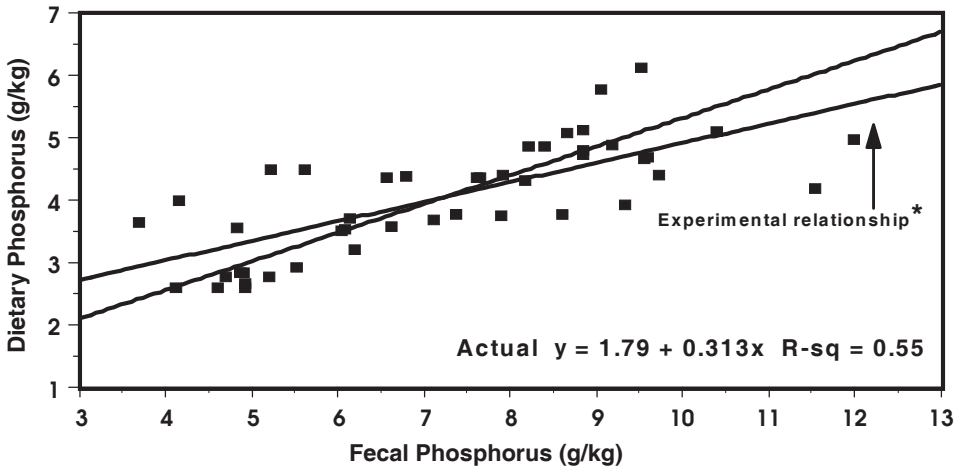

Figure 2. Relationships between fecal P and dietary P on 47 dairy farms in Wisconsin
(* experimental relationship from Zhigou Wu, personnel communication).

Labeling of Dairy Feces and Urine by Feeding Organic or Inorganic ^{15}N

J.M. Powell, G. Iemhoff and Z. Wu

Introduction

Approximately 60 to 70% of N consumed by a dairy cow is excreted in manure during lactation and 80 to 85% during the dry period. Nitrogen excretion by a cow can be divided into three pools: 1) urine N, 2) fecal endogenous N consisting of microbial products and microorganisms from the rumen, the intestine and the hind gut, and the N originating from the digestive tract itself, and 3) fecal undigested feed N. The proportion of urine N, endogenous N and undigested feed N in feces is approximately 55, 36 and 9% respectively for non-lactating dairy cows fed a forage diet (Powell and Wu, 1999).

The chemical form and mineralization rates of organic N in animal manure are not well understood. Rumen microbial and other endogenous, organic N forms in feces can provide a significant contribution to crop N requirements. When manure is applied to soil, urine N mineralizes rapidly, followed by fecal endogenous N and undigested feed N (Sorensen et. al., 1994; Somda et. al., 1995).

More accurate estimation of manure nutrient availability is needed if we are to expect farmers to improve manure management. The main objective of this study was to enrich dairy manure in ^{15}N for direct measurement of manure N mineralization in field and greenhouse trials and in soil/manure incubations. An additional objective was to label manure N components to test the hypothesis that urine N and endogenous fecal N applied to soil are the main N components of dairy manure available for crop uptake the first year after manure application. Labeling only urine and fecal endogenous N using inorganic ^{15}N would be much easier and less costly than labeling sufficient forage and subsequent feeding of this forage to cows to obtain uniformly labeled fecal N components. The undigested feed N in feces, having already undergone degradation by rumen microorganisms, appears to be recalcitrant in soil (Sørensen and Jensen, 1998).

Methods

Two methods were used to label dairy manure with ^{15}N. The organic method involved ^{15}N labeling of alfalfa (*Medicago sativa* L.) and corn (*Zea mays* L.) and the subsequent feeding of these forages to 2 dairy cows to label urine N, and endogenous N and undigested feed N in feces. The inorganic method involved the direct feeding of ^{15}N-labeled urea to 2 dairy cows to obtain ^{15}N-labeled urine N and endogenous fecal N. Ruminally-fistulated non-lactating dairy cows were used. For both methods, cows were adapted to a diet consisting of 55% alfalfa hay and 45% corn silage (dry matter basis) for 7 d; both unlabeled. For the inorganic treatment group, each cow was adapted to urea by feeding 100 g/d of urea (^{15}N at natural abundance) mixed with the forage. After the adaptation period, ^{15}N-enriched alfalfa hay and corn silage (4.824 atom % ^{15}N) were fed for 36 h to the organic treatment group. A single dose of 100g of 5 atom % ^{15}N-labeled urea was fed to each cow in the inorganic treatment group. All feces were collected from metal catchment containers and rubber bedding mats, and urine from catheters for 192 h after initial feeding.

The N contained in cell walls of feces (undigested feed) was determined as neutral detergent insoluble N (NDIN). Neutral detergent soluble-N (NDSN) in feces (microbial- and endogenous-N) was estimated as the difference between total N and NDIN (Mason and Federiksen, 1979). Total N and ^{15}N concentrations in feeds, feces and urine were determined using a Carlo Erba elemental analyzer coupled with a Europa 20/20 tracermass.

Results and Discussion

For cows fed ^{15}N-enriched forage, ^{15}N began to appear in urine between 4 to 8 h with peak ^{15}N concentrations occurring by 24 to 30 h (Fig. 1). ^{15}N began to appear in feces between 8 to 16 h with peak ^{15}N concentrations occurring between 30 to 60 h. For cows fed ^{15}N-enriched urea, ^{15}N began to appear in urine within 2 h and in feces within 8 h. Peak ^{15}N concentrations were attained between 6 to 8 h in urine and around 32 h in feces. The feeding of a single dose of 100 g of 5 atom % ^{15}N urea to each of two cows resulted in much lower enrichment of urinary and fecal N. The ^{15}N enrichment of manure components can be enhanced by prolonging the feeding of ^{15}N-labeled urea (Fernandez et. al., 1999). Future labeling using the inorganic method will involve daily doses of 100 g of 5 atom % ^{15}N urea for 4 to 6 days.

Potential differences in N mineralization necessitate the uniform ^{15}N labeling of fecal N components for nutrient cycling studies (Sørensen et al., 1994). Uneven ^{15}N labeling of fecal N components could result in error when estimating the rate and amount of fecal N mineralized in soil. Feces having a greater labeling of endogenous N than undigested feed N would appear to mineralize more rapidly in soil than feces having uniform labeling. Feces having a greater labeling of undigested feed N than endogenous N would appear to mineralize slower in soil than feces labeled similarly (Jensen et al., 1999). For cows fed ^{15}N-enriched forage, uniform 15N labeling of total fecal N and undigested feed N occurred at 48 h (Fig. 2). The proportionate combination of feces obtained before and after 48 h would, therefore, be necessary to obtain feces having uniformly-labeled N components.

Of the total ^{15}N fed, 56% was recovered by 192 h from cows

fed the ^{15}N-enriched forage diet and 59% from cows fed the ^{15}N-enriched urea diet (Table 1). The N recovered in feces from cows fed ^{15}N-enriched urea should have reflected only endogenous fecal N. Undigested forage N should not have been enriched in ^{15}N because only inorganic ^{15}N was fed. However, a detected ^{15}N enrichment of fecal NDIN from cows fed ^{15}N urea (data not shown) may suggest the presence of ^{15}N-enriched glycocalyx structures attached to neutral detergent fiber. This effect is being investigated further.

Conclusions

^{15}N-enriched forage must be fed to label all manure components (urine, endogenous fecal N and undigested feed N in feces). Feeding ^{15}N-enriched urea only labels urine and endogenous fecal N. ^{15}N-enriched dairy manure components may be selected for differential use in short- or long-term nutrient cycling studies depending on the ^{15}N enrichment level of urine and feces. Uniform labeling of fecal N components is necessary for accurate determination of manure N mineralization in soils. The results of this project may have several theoretical, as well as practical, implications for improving environmental impacts of dairy manure management. The proposed ^{15}N techniques may provide tools for better understanding nutrient flow in various components of the animal-feed-manure-soil/crop-environment continuum. This information could increase our confidence in manure nutrient availability to subsequent crops and provide the basis for developing alternative, economically viable and environmentally sound manure management practices. The relative effectiveness of using ^{15}N-labeled dairy manure in nutrient cycling studies will depend on their ability to more accurately measure N mineralization in soils than the classical, indirect measurements (e.g. fertilizer equivalent) currently in use.

Table 1. ^{15}N recovery in dairy manure components.

^{15}N labeling method	Manure component	% of ^{15}N fed
^{15}N-enriched forage	urine	31
	feces	25
^{15}N -enriched urea	urine	44
	feces	15

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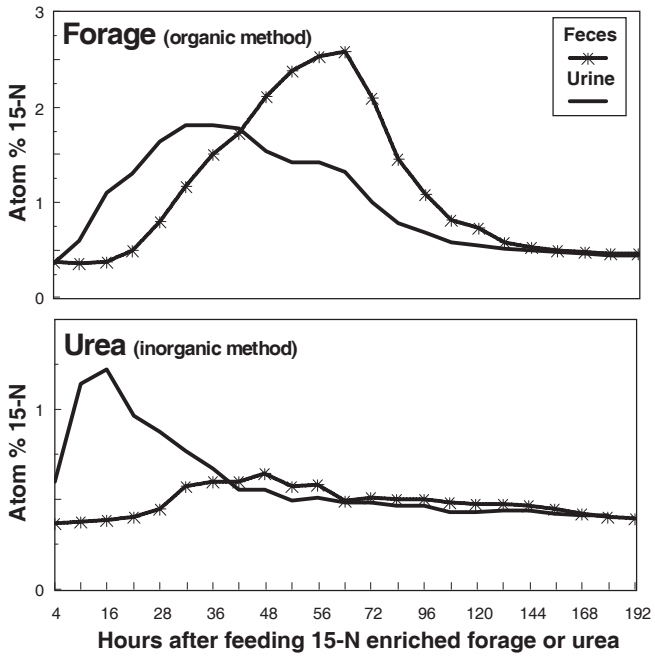

Figure 1. Pattern of ^{15}N excretion in dairy feces and urine after feeding ^{15}N enriched forage (Powell and Wu, 1999) or ^{15}N enriched urea (Powell et. al., 1999).

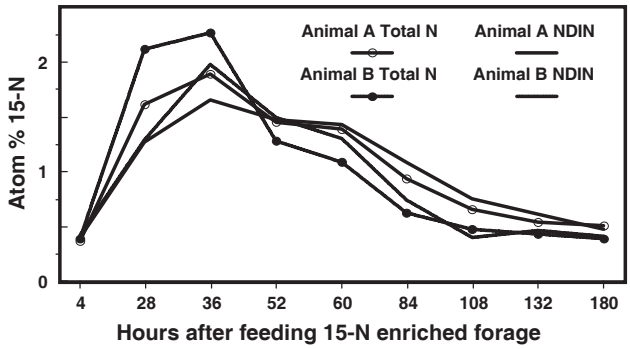

Figure 2. ^{15}N excretion in total fecal N and undigested feed N (NDIN).

Feeding a Complete Pelleted Calf Starter Containing Varying Levels of Alfalfa Leaf Meal to Holstein Calves

D.M. Allen, W.P. Hansen, H. Chester-Jones, J.G. Linn, and H.G. Jung

Introduction

Alfalfa leaf meal (ALM) is a potential byproduct of biomass energy production. The biomass process involves a mechanical separation of alfalfa leaves and stems. The alfalfa stems are used to produce electricity through a gasification process. ALM is the remaining fraction which primarily includes alfalfa leaves and fine stems. Earlier studies found ALM improved feed efficiency in beef calves consuming creep feeds containing ALM. The objective of this study was to determine if similar results may be realized for Holstein calves fed ALM as a portion of a complete pelleted calf starter.

Materials and Methods

Two studies were conducted. In study 1, forty-two calves from the St. Paul, MN dairy herd were blocked by sex and randomly assigned to one of three calf starters at 4 days of age. Starter treatments were: a commercial complete calf starter (0%), the commercial calf starter base with ALM replacing 12.5% of the dry matter (12.5%), and the commercial calf starter base with ALM replacing 25% of the dry matter (25%). The nutrient composition of the starters is shown in Table 1. Calves were fed their respective calf starter until 60 days of age. Calf starter intake was measured daily and body weight was determined weekly. Study 2 was conducted at the Southern Research and Outreach Center (SROC) in Waseca, MN and Northwest Research and Outreach Center (NWROC) in Crookston, MN. The study was a 3 x 2 factorial design with three milk replacers and two calf starters. The milk replacers contained 20% protein:20% fat with 0, 5, or 10% processed erthrocyte protein (PEP), which is a processed blood protein. The complete calf starters contained either 0% or 25% ALM with a similar ingredient composition as the starters used in Study 1 (Table 1). Thirty and forty calves were used for the study at the NWROC and SROC, respectively. Calves were blocked by sex and randomly assigned to one of six treatments at 4 days of age. Body weight and calf starter intake was determined weekly.

Results

In study 1, calves fed the 0% ALM calf starter consumed a greater amount of feed from 4 to 60 days of age than calves fed the other starters. Calves fed the 25% ALM calf starter consumed 8.7% less starter than calves fed the 0% ALM starter. Average daily gain (ADG) was higher for calves fed the 0% starter. Feed efficiency decreased by 5% and 6% for calves fed 12.5 and 25% ALM starter compared with the 0% ALM starter. These results are not similar to results from the first year of the beef creep feed study. Calves fed the ALM creep feed consumed less feed than calves fed the control creep feed (2.72 vs. 4.99 kg/day, respectively) but had similar average daily gain (2.67 vs. 2.63 kg/d, respectively). Based on these results, feed to gain was improved from 1.89 kg/kg for calves fed the control to 1.02 kg/kg for the ALM fed beef calves. It has been hypothesized that an improvement in rumen development may be the reason for improved feed efficiency for beef calves on ALM treatments. No significant ($P > 0.05$) interactions between milk replacer and calf starter treatment were found in Study 2. Calf performance was similar between Studies 1 and 2. Calves fed the control calf starter consumed 3.9 kg more starter than calves fed the 25% ALM starter from 4 to 60 days of age. A similar trend in feed efficiency was found for Study 1 and Study 2. Calves fed the 0% ALM calf starter showed improved feed efficiency over calves fed the 25% ALM calf starter. Environment had a dramatic effect on feed intake. Calves on trial at the research and outreach centers were housed inside in individual calf crates for the entire trial whereas the St. Paul calves were fed outside in individual hutches throughout the months of November 1998 to February 1999.

Conclusions

Alfalfa leaf meal has potential as a source of fiber and protein in complete pelleted calf starters. Intakes of starters containing ALM was reduced, but calf performance was similar between conventional and ALM containing starters. While the improved feed efficiency observed when ALM was fed as a creep supplement to beef calves was not seen with Holstein calves, performance of the dairy calves was acceptable. Availability of ALM for livestock feeding will depend on successful development of an alfalfa-based biomass energy system.

Table 1. Nutrient composition of calf starter treatments[1].

	% ALM in starter DM				
	Study 1			Study 2	
	0%	12.5%	25%	0%	25%
DM, %	91.4	91.1	91.2	91.4	91.2
	- % DM - - - - - - - - - - - - - - - - - -				
CP	18.1	17.0	17.2	20.0	21.6
NDF	34.7	35.1	35.6	35.4	33.7
Ether Extract	2.1	2.7	2.7	3.6	4.4
Ash	8.9	7.8	9.8	9.7	10.3
Ca	1.72	1.23	1.69	1.72	1.69
P	0.43	0.57	0.54	0.43	0.54
Mg	0.36	0.34	0.33	0.36	0.33
K	1.50	1.21	1.21	1.50	1.21

[1]Treatments were: 0% = commercial complete calf starter, 12.5% = calf starter containing 12.5% ALM, 25% = calf starter containing 25% ALM.

Table 2. Calf starter intake, average daily gain (ADG), and feed efficiency (F/G) of calves from 4 to 60 days of age fed ALM calf starter[1].

	% ALM in starter DM				
	Study 1			Study 2	
	0%	12.5%	25%	0%	25%
Starter Intake, kg	64.8	62.9	59.6	33.9	30.0
ADG, kg	0.55	0.50	0.46	0.51	0.46
F/G, kg/kg	2.63	2.75	2.79	2.04	2.12

[1]Treatments were: 0% = commercial complete calf starter, 12.5% = calf starter containing 12.5% ALM, 25% = calf starter containing 25% ALM.

Sources of Varitation in Protein Degradation Rates in the Inhibitor In Vitro System

G.A Broderick, M.L. Murphy, P. Uden & A. Lapins

Introduction

Information of rates and extents of ruminal degradation of feed proteins is required by a number of the new systems of ruminant ration formulation. The lack of reliable data on protein degradation causes dairy farmers to feed extra protein to counter real or imagined insufficiencies of absorbable protein. To avoid problems due to protein over-feeding, routine methods that are both accurate and rapid must be available to allow timely characterization of the protein degradation of common feeds. We have devoted a number of years to developing the inhibitor in vitro (IIV) method for assessing protein degradation. In this method, metabolic inhibitors are added to ruminal inoculum to allow quantitative recovery of protein breakdown products. Rate and extent of degradation are computed from the time-course of appearance of ammonia and total free amino acids (TAA). Although the IIV method has proven valuable for several applications, degradabilities have varied considerably among incubations, even when using inocula from the same donor animals fed the same diets. This variation has necessitated greater replication, both within and among incubations. Understanding and controlling sources of variation are required to reduce unnecessary replication and to improve reliability of estimates of protein degradation.

Materials and Methods

The basic IIV method (Broderick, Brit. J. Nutr. 58:463-475, 1987) was used to estimate ruminal degradation rates for three standard proteins: casein, solvent soybean meal (SSBM) and expeller soybean meal (ESBM). Inocula were obtained from ruminally cannulated donor cows fed either a maintenance diet of all grass silage or a lactation diet containing grass silage plus concentrate (a mixture of barley, oats, sugarbeet pulp, rapeseed meal and soybean meal). Fresh ruminal contents were transported to the laboratory and large feed particles removed by filtering through a cylindrical wire screen and then through 4 layers of cheesecloth. Aliquots of filtered inocula were centrifuged (10,000 x g, 30 min., 4°C) and pellets analyzed for dry matter (DM) and N. $NaHCO_3$ (0.12 M), or an equimolar mixture of NaH_2PO_4 and Na_2HPO_4 (0.12 M), plus a carbohydrate mixture (/l, 8 g maltose, 4 g sucrose, 4 g xylose, 4 g pectin, 4 g soluble starch) were added and inocula were pre-incubated for 4 hours. At 0-time and every hour, samples were taken for analysis of ammonia and TAA and pH was measured; if pH was < 6.2, pH was adjusted up to 6.4 with NaOH. After pre-incubation, inhibitors (1.5 mM hydrazine and 45 mg/ml of chloramphenicol) plus 3 mM mercapto-ethanol were added to the inoculum 30 min. before starting the incubation. Protein sources (4.0 mg N/tube) were weighed into 50-ml glass centrifuge tubes; 10 ml of warm (39°C) McDougall's buffer was added to each tube to wet the proteins 1 h prior to starting the incubation. Incubations were begun by adding 20 ml/tube of inoculum (final protein concentration was 0.133 mg N/ml); tube headspace was gassed with CO_2. Tubes were capped with bunsen valves, swirled and placed in a 39°C water bath; tubes were re-swirled at 1-hour intervals. Incubations were run for 4 or 6 hours and were stopped by adding TCA (5% wt/vol). Ammonia (phenol-hypochlorite assay) and TAA (ninhydrin assay) were determined in TCA supernatants using AutoAnalyzer methods.

Results and Discussion

Compared to the mixture of NaH_2PO_4 and Na_2HPO_4, buffering pre-incubations with $NaHCO_3$ increased observed degradation rates for casein and SSBM, possibly because the bicarbonate was more effective in maintaining pH and microbial growth. Mean background NH_3 concentration was reduced from 9.0 to 0.7 mM by pre-incubating. Effect of feed intake of donor animals, and of time after feeding of inoculum sampling, on observed degradation rate is in Table 1. Casein rate nearly doubled using inocula from lactating versus non-lactating cows with energy and DM intakes that were less than half as great. Degradation rates observed for both SSBM and ESBM also were more rapid, but their rates were increased only by an average 35%. Degradation rate for casein was increased after feeding; rate with inocula taken 4 after feeding was 31% more rapid than that with inocula taken right before feeding (Table 1). The opposite effect was observed with the soybean meals—degradation rates were slowest with inocula obtained 4 h after feeding. Thus, there was a significant ($P < 0.001$) interaction of protein and time after feeding on IIV degradation rates.

The relationship of inoculum DM content (before pre-incubation) to degradation rates for the three proteins was assessed in the series of experiments with the dry and lactating donor cows. Rates observed for casein were highly correlated ($r^2 = 0.81$) to DM content, a measure of microbial mass in the inoculum; however, there was no relationship of degradation rate to microbial biomass for the two soybean meals (Figure 1). A strong correlation also was found between casein rate and inocula concentrations of TAA ($r^2 = 0.68$) but not NH_3 ($r^2 = 0.27$). Previously, we had found that most of the free TAA in ruminal fluid was associated with the intracellular space while most NH_3 was extracellular. Inocula TAA concentrations probably served as a measure of microbial protoplasm. The degradation rates observed for the soybean meals were unrelated to TAA or NH_3 concentrations ($r^2 = 0.10$). Thus, it appeared that different factors influenced degradation rates for the largely soluble protein casein and those for SSBM (with about 25% soluble protein) and ESBM (with < 5% soluble protein). Previously, peptide accumulation was found to occur during

the early phases casein degradation but not with degradation of either soybean meal. We speculate that inocula characteristics influencing peptide catabolism may account for the some of this dissociation between casein and soybean meal degradation.

There was a tendency for slower degradation rates to be observed for soybean meals in incubations conducted in Sweden then in earlier incubations at the Dairy Forage Center. Therefore, an analysis was done of degradation results obtained for the same samples of casein, SSBM and ESBM at both locations (Table 2). The overall mean rate for casein in Wisconsin was only 80% of that found in Sweden; however, the range of rates in Wisconsin (0.10-0.42/h) was similar to that in Sweden (0.11-0.37/h). Difference in means may be explained by a greater number of incubations with lower activity in the Wisconsin data set and not due to inherently more rapid casein degradation with Swedish inocula. Greater differences were seen for SSBM; this protein was degraded only 56% as fast under Swedish conditions. Although ESBM degradation rates were not different by location, there were fewer replicates in the ESBM data set. Blank NH_3 concentrations tended to be higher, even after pre-incubation, in Swedish inocula. Therefore, we tested whether end-product accumulation may have contributed to the slower rates for soybean meal.

Diluting the medium to reduce end-product concentrations had no effect on degradation rate of any of the proteins (Table 3, upper panel); however, diluting the medium also diluted the reactants (the microbial degradative enzymes and substrate proteins). Adding N end-products as a mixture of NH_3, free TAA and peptides reduced degradation rates for casein and SSBM, but not ESBM (Table 3, lower panel). This suggested that at least some the apparent variation in observed degradation rates may to due differences in the amounts of end-product that accumulate in different incubations.

Summary and Conclusion

Several potential sources of variation in protein degradation rates were identified in a series of IIV experiments. Microbial biomass content of the inoculum had a greater influence on rates of degradation of casein than of soybean meals. Increasing feed intake of rumen donor animals increased degradation rates for casein and soybean meals but the effect was greater on casein. The effect of time of inoculum sampling relative to feeding was contradictory for these proteins: casein rates increased while soybean meal rates declined with time after feeding. The much slower rates observed for SSBM in Sweden relative to Wisconsin may be related to a greater depression of activity due to end-product inhibition in the Swedish studies.

Table 1. Effect of donor energy intake and time after feeding of obtaining inocula on observed rates of protein degradation.

	Level of intake[1]			
NE_L, Mcal/d	13.4	38.8		
CP, kg/d	1.24	3.74		
Protein	Dry	Lactating	SEM	$P > F^2$
	(/h)			
Casein	0.115	0.213	0.011	< 0.001
Solvent soybean meal	0.043	0.059	0.002	< 0.001
Expeller soybean meal	0.015	0.020	0.001	< 0.001
Overall mean	0.058	0.098	0.003	< 0.001

	Time after feeding (h)				
Protein	0	2	4	SEM	$P > F^2$
		(/h)			
Casein	0.143[b]	0.161[ab]	0.188[a]	0.010	0.010
Solvent soybean meal	0.061[a]	0.048[b]	0.044[b]	0.002	< 0.001
Expeller soybean meal	0.021[a]	0.016[b]	0.015[b]	0.001	< 0.001

[1]Mean DM intakes of two dry and two lactating cows were, respectively, 8.7 and 21.0 kg/d.

[2]Probability of significant effects of level of intake (top panel) and time after feeding (bottom panel) on observed degradation rate.

[a,b]Mean degradation rates in lower panel with differing superscripts are different ($P < 0.05$).

Table 2. Effect of location of IIV incubation on observed protein degradation rates.

Protein	Location (n)[1]	LS Mean	SEM	$P > F^2$
		(/h)		
Casein	Wisconsin (102)	0.207	0.005	< 0.001
	Sweden (36)	0.258	0.008	
Solvent SBM	Wisconsin (92)	0.119	0.005	< 0.001
	Sweden (32)	0.067	0.008	
Expeller SBM	Wisconsin (73)	0.029	0.006	0.355
	Sweden (18)	0.017	0.011	
Location (all three proteins)				0.492
Location x Protein				< 0.001

[1]Number of IIV incubations conducted at each location in determining mean degradation rate.
[2]Probability of significant effect of location, and location-by-protein interaction, on observed degradation rates.

Table 3. Possible effects of end-product accumulation on observed rates of protein degradation.

	Medium dilution[1]			
Protein	1X	2X	SEM	$P > F^2$
	(/h)			
Casein	0.238	0.232	0.014	
SSBM	0.055	0.057	0.004	
ESBM	0.017	0.021	0.002	
Overall mean	0.104	0.103	0.008	0.970

	End-product addition[3]			
Protein	0	+	SEM	$P > F^2$
	(/h)			
Casein	0.327	0.283	0.013	< 0.001
SSBM	0.059	0.046	0.006	0.013
ESBM	0.018	0.020	0.002	0.641
Overall mean	0.135	0.117	0.002	< 0.001

[1]Extent of dilution of incubation medium; 1X = undiluted and 2X = dilution of medium to twice its normal volume by addition of McDougall's buffer.
[2]Probability of significant effects of medium dilution (top panel) and end-product addition (bottom panel) on observed degradation rates.
[3]Nitrogen added to "+" vessels as NH_4SO_4 and enzymatic- and acid-hydrolyzed casein increased mean background concentrations of NH_3 from 1.0 to 5.1 mM and of TAA from 1.9 to 2.6 mM at the start of the incubation.

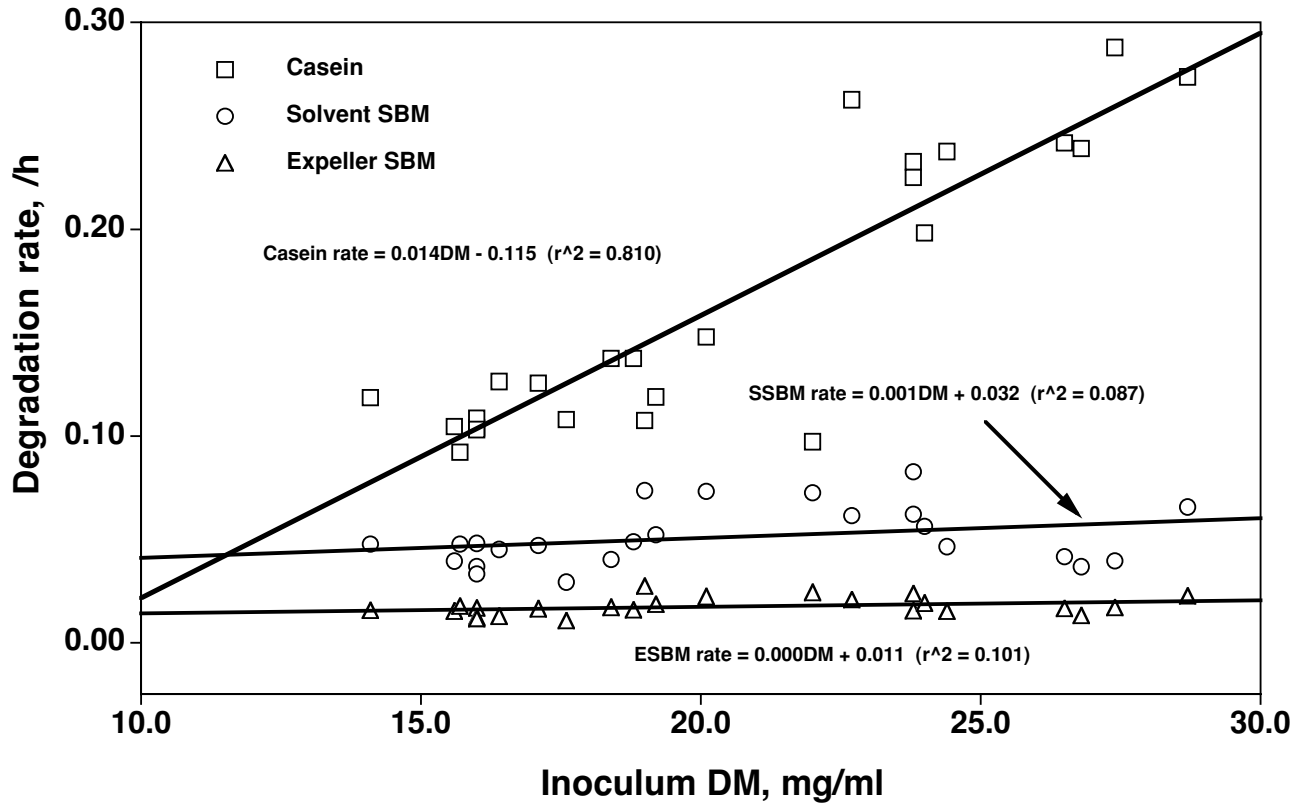

Figure 1. Effect of inoculum DM content on protein degradation rates observed for casein, solvent soybean meal (SSBM) and expeller soybean meal (ESBM).

Farm/Herd Report-Wis.

U.S. Dairy Forage Research Center - Annual Dairy Operations Report
January 2000
L.L. Strozinski – Herd Manager

HERD STATISTICS			CHANGE FROM PREVIOUS YEAR
Herd Inventory			
Milking cows		297	-17
Dry cows		52	+11
Average cow age	48 months		-2
Percent first lactation	46%		+11
Percent second lactation	24%		-5
Percent third lactation	15%		-2
Percent greater than third	14%		-3
Herd replacements		336	-3
Total		**685**	-9
Rumen Fistulated Cows	16		-6
Herd Performance			
Cows calved		362	-29
Heifer calves born live		164	-17
Heifer calves born dead		10	-15
Bull calves born live		185	+16
Bull calves born dead		28	-11
Heifer calves died < 1 year old		1 (0.6%)	-1
DHIA rolling herd average			
Milk		22,547 lbs.	+658
Protein		719 lbs.	+27
Fat		835 lbs.	+25
Milk sold in 1999		7,415,632 lbs.	+429,277
Heifer calves sold		11	-3
Bull calves sold		185	+16
Cows sold		153	+47
Cows culled for:			
Reproduction problems		35	+10
Poor production		20	+11
Poor udder		23	+4
Poor feet and legs		10	+1
Mastitis		41	+12
Other		24	+9
Cattle sales revenue		$76,203.30	+13,727.78
Herd Reproduction			
Average days open		123	+3
Average calving interval		12.95 months	0
Average services per conception		2.4	+0.3
Average age at first calving		24 months	0

Once again it was a very good year at the farm in regards to the weather, crops, performance of the herd and research output. Numbers of both milking age animals and herd replacements have remained relatively constant over the year. Keeping the herd size constant has allowed us to do additional culling of the herd to improve production average, health and overall quality of the cattle. Increased culling is reflected in the increase in cattle sales revenue and the decline of the average age of our milking animals from 46 to 44 months. It is also reflected by the rise in the percentage of animals in the milking herd that are in their first lactation. Our first calf heifers are doing very well and with calf mortality at only one for the year, we have a large promising group of replacements for the future. Our DHIA rolling herd average for milk missed the 23,000 pound mark by 60 pounds in September. Hopefully we will pass that goal in 2000. The farm sold more than 7.4 million pounds of milk in 1999. The farm "mailbox" net price received per hundredweight ranged from $12.15 to $17.93 in 1999. The average price received was down by $2.02 to $14.46. Milk price outlook for 2000 is not good.

Once again, a number of cows in our herd have received special recognition by the Holstein Association and DHIA. The bull calf we sold to Accelerated Genetics in 1998 is now in their young sire-sampling program. We will be using some of his semen back in our herd in 2000.

The dam of that bull (now scored 85) continues to do very well in the herd. She completed a record of 32,370 pounds of milk in 310 days in 1999. This past December we flushed this cow and transferred ten embryos to recipients within the herd.

Several facility and program improvements were made in 1999. Improved pasture lanes were constructed in the grazing area. Self-locking head gates were installed in the mature cow free stall barn. These headlocks have proven to be a real time saver. In 1999 we changed from using paper towels to using cloth towels to wipe cows udders prior to milking. A washer and dryer were purchased to launder the towels daily. This change was made in an effort to improve cow preparation, improve milk quality and reduce the incidence of mastitis.

The largest and most challenging task in the dairy operation during 1999 was recruiting and retaining quality labor. During a large portion of the year, the dairy operated with a labor shortage. Assistance from the field crew and student labor during the summer helped to fill the gaps but many of the planned extra projects were not completed. Hopefully the next wage plan will reduce this problem. Currently the dairy crew is fully staffed.

U.S. Dairy Forage Research Center - Annual Field Operations Report
January 2000
R.P. Walgenbach – Management Agronomist and Farm Manager

The 1999 crop year was similar to the exceptional crop year of 1998. The spring of 1999 began with a dry and warm March. It followed a relatively mild winter and as in 1998 resulted in an early thaw of frozen soils. These conditions and the low rainfall in March (Table 1) resulted in a very early planting of barley and alfalfa (Table 2). This was fortunate because the weather during April produced 8.5 inches of rain for the month. The moderate winter did little damage to over wintering of alfalfa. Alfalfa grew and developed more rapidly than alfalfa does during this growth period in most years; however, it did not grow and develop as fast as alfalfa did in 1998. In 1998 the first cutting was made on 16 May, while this cutting was made on 21 May in 1999 (Table 3). This early start to cutting set the stage for four growing season harvests. Early season rains provided potential for high yields of Dry Matter (DM). But unlike 1998, rain was not as plentiful in August and September. The most significant infestation of leafhoppers in the last ten years also contributed to lower dry matter accumulation in alfalfa. All alfalfa fields were sprayed for leafhoppers during second and third growth periods. The timing and/or amounts of insecticide used did not provide adequate control of leafhoppers in some fields this season. As I stated in last years report we continue to use surface tillage via the Aerway tool especially where manure has been applied. This excellent growing season with early season moisture and above average temperatures produced very high yields of barley, corn and soybeans (Table 4). The 93.7 bushels per acre average yield of barley and the 183.1 bushels per acre average yield of corn grain are the highest we have produced at the research farm. While average yield of soybeans were excellent 63.5 bushels per acre, the 1998 crop produced a higher average yield. I do believe that both corn and soybean yields were somewhat reduced due to the dry conditions in September. In two small fields a considerable amount of white mold caused yield reductions. A few cornfields had obvious symptoms of compaction caused by trucks spreading manure when soil was too wet. We have carryover of 1998 harvested forage stored in bags inside the Badger Army Ammunition Plant (BAAP). Because of this carryover and some additional forage in 1999, I am planning on reducing forage acres in the 2000 crop year. We have moved a couple of bags into bunkers at the farm and will continue to do so until we use most of this carryover of alfalfa.

This past season the field crew completed the fencing project started in the autumn of 1998. They also completed a laneway to the area of the pasture containing three small watersheds. These watersheds will be used to evaluate the impact of over wintering heifers on snowmelt runoff in the spring. A grain bin for storing soybeans has been purchased along with an unload system that will allow us to automate our soybean roasting system. When the bin is installed we will unload harvested beans into the storage bin and from this bin beans will be augered directly to a container that feeds the soybean roaster. Another facility improvement made this winter was to install a plastic lining molded to fit the groove for the chain that pulls scrappers in F and G barns. This should reduce wear on the chain, reduce the energy required to run the chain and prevent the chain from freezing in the groove during sub freezing temperatures.

Dean Borcherding was hired as a Farm Equipment Operator in April 1999 as a replacement for Mike Rogers. Dean was a self-employed dairy farmer and after selling his farm spent some time working on a large dairy herd and a horse ranch in Arizona. Dean is an enthusiastic and very hard working employee. We are glad to have him with us.

In last years report I commented that the General Service Administration (GSA) had planned to have a preliminary reuse plan for the Badger Army Ammunition Plant available for public comment by early summer 1999. But before GSA officials released a preliminary plan the Sauk County Board with the support of Congresswoman Tammy Baldwin and Senators Kohl and Feingold asked the GSA officials to delay issuing a preliminary reuse plan to give county and local governments additional time to study the issues of concern to local communities. The GSA officials agreed to delay issuing a reuse plan. GSA officials at the same time also insisted that planning at the local level continues and that they be included in planning activities. Since 2/3 of the BAAP land is located in the town of Sumpter and the town of Sumpter is required by the county to come up with a land use plan for the township, they have undertaken the task of developing a plan to include lands of BAAP that will ultimately be in the township. A committee was formed by the township to collect information, meet with potential property owners and managers, and to come up with a potential zoning and land use plan. Congresswoman Baldwin also has obtained a $100,000 grant for Sauk County from the Department of Labor to help the county in a facilitative process. As one might imagine there are strong ideas and feelings about how the property should ultimately be used. The grant will be used to hire a facilitator who will try and develop a consensus among parties who have a strong interest in how the BAAP is to be used. This process should begin sometime in the spring of 2000. The USDFRC, Ho-Chunk Nation, Wisconsin Department of Administration and Natural Resources, Local Rail Commission, the Community Conservation Coalition for Sauk Prairie as well as others will form a steering committee to work with a facilitator toward reuse consensus that will hopefully have the support

of the local communities. USDA-ARS and USDFRC personnel have been working very diligently with GSA personnel to keep the transfer of BAAP lands (1720 acres) to the USDA-ARS on track.

I repeat my comments from last year that USDA's acquisition of pasture and cropland from the excessed BAAP are critical for the continuation of the USDFRC research programs. We appreciate, are encouraged by, and look forward to continued support from the dairy forage producers; industry representatives and organizations; and the College of Agricultural and Life Sciences, University of Wisconsin-Madison as we seek to acquire the resources needed to maintain this important and productive research program.

This has been another extremely busy and quite remarkable year at the USDFRC. It produced record crop yields, a variety of forage research materials successfully harvested and stored, some major improvement projects completed and a continued opportunity to acquire the land needed to support our research programs. The USDFRC farm has an excellent staff of employees that contribute to a productive and successful research program. I thank all of our employees for their efforts this past year.

Table 1. 1999 precipitation (ppt)

Month											
Jan*	Feb*	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
ppt inches											
		0.38	8.52	3.34	4.66	5.0	2.49	1.53	1.2	1.03	0.90

*ppt was not recorded for these months

Table 2. 1999 planting and harvesting dates.

		Planting		Harvesting	
Crop	Acres	Start	Finish	Start	Finish
Barley	98.8	03/29	03/31	07/13	07/22
Soybeans	227.3	05/03	05/20	10/06	10/20
Corn Grain	296.2	04/19	05/11	09/16	10/15
Corn Silage	134.4	04/19	06/20	08/25	10/12
Alfalfa – Spring	63.0	04/01	04/02	—	—
Alfalfa†- Summer	66.0	08/09	08/11	—	—
Winter Wheat‡	112.0	10/07	10/12	—	—

†Planted no-till after barley
‡Planted no-till after soybeans

Table 3. 1999 forage cutting dates.

	Alfalfa - Established			Alfalfa - Spring Seeded		
Crop	Acres	Start	Finish	Acres	Start	Finish
First	312	05/21	05/28	63	06/21	06/24
Second	312	06/23	07/08	63	07/24	07/25
Third	312	07/26	08/03	63	08/28	08/29
Fourth	100	09/01	09/20			

Table 4. 1999 crop yield data.

		Yields			
Crop	Acres	Low	High	Mean	Total
		- - - - - - - bushels per acre - - - - - - - - -			
Barley	98.8	62.9	101.4	93.7	9,252
Soybeans	227.3	49.1	68.5	63.5	14,428
Corn Grain†	296.2	148.1	209.2	183.1	54,233
		- - - - - - -tons DM (as is)/acre - - - - - - -			
Corn Silage DM	134.4	5.5	8.6	7.1	955
Corn Silage (As Is)‡		(16.7)	(27.9)	(20.3)	(2,730.3)
Alfalfa††	249.0	3.20	5.38	4.27	1,064
Alfalfa Spring Seed	63.0	—	—	3.26	205.4
Total	1068.7				

†15,104.6 bushels were harvested and stored as dry shell corn. Moisture range at harvest for dry shell corn was 17.3 to 21.8% and for high moisture shelled corn it was 24.0 to 32.0%. Corn yields are adjusted to 15% moisture.
‡Corn silage harvest moisture ranged from 69 to 55%.
††Alfalfa yields include hay and alfalfa silage.

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**Publications that appeared too late to include in last year's report.